

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

THIS PAGE BLANK (USPTO)

PCTWORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁵ : A61K 9/12, 31/725	A1	(11) International Publication Number: WO 93/19734 (43) International Publication Date: 14 October 1993 (14.10.93)
(21) International Application Number: PCT/US93/02880 (22) International Filing Date: 26 March 1993 (26.03.93) (30) Priority data: 07/862,448 2 April 1992 (02.04.92) US (71) Applicant: BAKER NORTON PHARMACEUTICALS, INC. [US/US]; 8800 N.W. 36th Street, Miami, FL 33178 (US). (72) Inventor: AHMED, Tahir ; 3705 Granada Boulevard, Coral Gables, FL 33134 (US). (74) Agent: SCHIFFMILLER, Martin, W.; Kirschstein, Ottinger, Israel & Schiffmiller, 551 Fifth Avenue, New York, NY 10176-0024 (US).		(81) Designated States: AU, CA, FI, JP, KR, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: METHOD AND COMPOSITION FOR TREATING ANTIGEN-INDUCED AND EXERCISE-INDUCED ASTHMA (57) Abstract A method of treating a patient suffering from antigen-induced or exercise-induced asthma comprising the intrabronchial administration to the patient of an inhalant composition containing about 300 to about 2,000 units of heparin per kilogram of patient body weight in each dose. Commercial heparin, low weight heparins or heparin fragments and partially N-desulfated heparins or other non-anticoagulant heparins may be used. Suitable inhalant compositions for use in the novel treatment method are also provided.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LJ	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TG	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

**METHOD AND COMPOSITION FOR TREATING
ANTIGEN-INDUCED AND EXERCISE-INDUCED ASTHMA**

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to methods of preventing and reversing the symptoms and manifestations of asthma.

2. Description of the Prior Art

Chronic asthma can be considered to be predominantly an inflammatory disease with associated bronchospasm. The degree of reactivity and narrowing of the bronchi in response to stimuli is greater in asthmatics than in normal individuals. Persistent inflammation is responsible for the bronchial hyperreactivity. Mucosal edema and mucus plugging and hypersecretion may be present; pulmonary parenchyma is normal. Airway narrowing may reverse spontaneously or with therapy. Type 1 (immediate) immune responses may play an important role in the development of asthma in children and many adults; however, when onset of disease occurs in adulthood, allergic factors may be difficult to identify. Exposure to cold dry air, exercise and other aggravating factors also may trigger asthma.

The most common symptoms of asthma are breathlessness and chest tightness; wheezing, dyspnea, and cough also are prominent. Reduced pulmonary function typical of obstructive rather than restrictive airway

disease is usually observed. Asymptomatic periods often alternate with paroxysms.

Of the known triggers of asthma, allergens and exercise have received the most attention. Both are powerful, naturally occurring stimuli; exercise is a potential factor in the daily life of every asthmatic, whereas allergens only effect some persons with asthma. Nevertheless, more is known about the effects of antigen, and it is only in the last several years that interest in the pathophysiology and treatment of exercise-induced bronchoconstriction (EIB) has developed. The bulk of current evidence demonstrates that the essential stimulus for the development of the bronchoconstriction is a fall in the temperature of the airways secondary to heat and water loss, that occur during the conditioning of inspired air. If heat loss and airway cooling are prevented, obstruction to airflow does not develop, and if they are augmented by lowering the inspired air temperature and/or water content, or by increasing the level of ventilation, the obstruction proportionately worsens.

Despite these developments, it is not yet known how airway cooling produces its effects. There are a series of observations in the literature which suggest that mediators of immediate hypersensitivity may play a role. Although the direct effect of physical stimuli like cold on primed asthmatic airway smooth muscle has been proposed by

some, others believe that these physical stimuli are capable of degranulating mast cells, thus suggesting a central role of mast cell mediators in EIB. This however remains controversial. Recently, it has been demonstrated that leukotriene antagonists may attenuate EIB, thus underscoring the importance of leukotrienes and not histamine release from mast cells during EIB.

The general goals of drug therapy for asthma are prevention of bronchospasm and long-term control of bronchial hyperreactivity. Because it is usually not possible for either patient or physician to predict when bronchospasm may occur, patients with all but the most episodic and/or entirely seasonal attacks may require continuous therapy.

Beta agonists are useful; they stimulate beta₂-adrenergic receptors, increase intracellular cAMP, and inhibit the release of inflammatory mediators. Other useful drugs include theophylline and related xanthine drugs, which produce bronchodilation through unknown mechanisms; the bismolone, cromolyn, which prevents the release of mediator substances and blocks respiratory neuronal reflexes, and corticosteroids, which primarily decrease inflammation and edema. Anticholinergic drugs may relieve bronchospasm by blocking parasympathetic cholinergic impulses at the receptor level. Antihistamines occasionally prevent or abort allergic asthmatic episodes,

particularly in children, but they can only be partially effective in asthma because histamine is only one of many mediators.

Standard treatment for exercise-induced asthma includes the use of an inhalant. Beta₂-adrenergic agents administered 15 minutes before exercise provide some protection from broncho-constriction for up to four hours, and cromolyn sodium administered up to two hours prior to exercise may afford limited protection in some patients.

The current drug modalities used for treatment of allergy-induced and exercise-induced asthma suffer from a number of drawbacks. In general, the conventional agents have a relatively short duration of action and must be repeatedly administered for prophylaxis. Moreover, because of serious adverse effects associated with the use of agents such as beta₂-adrenergic agonists and corticosteroids, the therapeutic margin of safety with such agents is relatively narrow and patients using them must be carefully monitored. In the case of exercise-induced asthma or bronchoconstriction, the current treatment regimens are inadequate and often of only limited prophylactic value.

SUMMARY OF THE INVENTION

It is an object of the present invention to

provide a method and compositions for treatment of both antigen-induced and exercise-induced asthma which do not suffer from the drawbacks of the prior art.

It is a further object of the present invention to provide a method and compositions for the treatment of asthma which have long-term prophylactic effects and are also effective in reversing the manifestations of an asthmatic episode.

Still another object of the present invention is to provide a method and compositions as described above which are highly effective in preventing the onset of exercise-induced asthma.

Yet a further object of the present invention is to provide a method and compositions as described above which are safe, simple and relatively inexpensive.

In keeping with these objects and others which will become apparent hereinafter, the invention resides in a method of treating a patient suffering from antigen-induced or exercise-induced asthma through the intrabronchial administration to the patient of a pharmaceutical composition comprising from about 300 to about 2,000 units of heparin per kilogram of patient body weight in each dose, with about 1 to 4 doses administered daily. Novel inhalant heparin compositions are also provided in the form of liquid or powdered nebulizer or aerosol compositions containing suitable concentrations of

heparin.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a bar graph showing the effect of increasing doses of inhaled heparin on bronchoconstriction, with data given as mean \pm SE% increase SR_L (specific lung resistance).

FIG. 2 is a bar graph comparing the effects of benzyl alcohol, dextran sulfate and N-desulfated heparin on antigen-induced bronchoconstriction. The open bars represent control data, while post-drug treatment data are shown in hatched bars.

FIG. 3 is a bar graph comparing the effects of inhaled heparin on bronchoconstriction responses induced by antigen, compound 48/80 and histamine.

FIG. 4 is a bar graph showing the time-dependent inhibition of antigen-induced bronchoconstriction by inhaled heparin (1,000 units/kg). Data are shown as mean \pm SE. Starred values are significantly different from control ($p < 0.05$).

FIG. 5 includes four graphs showing the in vitro effects of heparin on antigen-induced tracheal smooth muscle contraction in sheep:

5A - Shows the inhibition of antigen-induced contraction by increasing doses of heparin; the tension is plotted as a percent of the acetylcholine maximum reflected

in 5C.

5B - Shows comparative inhibition by heparin (H) and nedocromil (NED) of antigen-induced tracheal muscle contraction.

5C - Shows failure of heparin to modify acetylcholine-maximal tension.

5D - Shows failure of heparin and nedocromil to modify acetylcholine-tracheal muscle contraction as shown by EC_{50} .

FIG. 6 includes bar graphs reflecting the effects of inhaled heparin (1,000 units/kg) on exercise-induced bronchoconstriction in 3 human subjects, with data shown as percent decrease in specific airway conductance (SG_{aw}).

DETAILED DESCRIPTION OF THE INVENTION

Heparin, a sulfated mucopolysaccharide, is synthesized in mast cells as a proteoglycan and is particularly abundant in the lungs of various animals. Heparin is not a specific compound of fixed molecular weight but is actually a heterogenous mixture of variably sulfated polysaccharide chains composed of repeating units of D-glucosamine and either L-iduronic or D-glucuronic acids. The average molecular weight of heparin isolated from animal tissues ranges from about 6,000 to about 30,000 Da.

Pharmacologically, heparin is known primarily as an anticoagulant. This activity results from heparin's

ability to bind to some of the residues of antithrombin III (AT-III), accelerating the neutralization by AT-III of activated clotting factors and preventing the conversion of prothrombin to thrombin. Larger amounts of heparin can inactivate thrombin and earlier clotting factors, preventing conversion of fibrinogen to fibrin.

The anticoagulant activity of heparin is related to the molecular weight of its polysaccharide fragments; low molecular weight components or fragments (for example, fragments having a molecular weight of less than 6,000) have moderate to low antithrombin and hemorrhagic effects. Similarly, low molecular weight heparins isolated from animal tissue have reduced anticoagulant properties because they consist primarily of the lower molecular weight fragments or fractions.

Commercial heparin, which is generally derived from beef lung or pork intestinal mucosa, has an average molecular weight of about 15,000-17,500.

It has now been discovered, surprisingly, that heparin administered intrabronchially is a potent and long-acting inhibitor of bronchospasm and bronchoconstriction and is consequently of great value in preventing and treating asthmatic episodes. Accordingly, the method of the present invention comprises the intrabronchial administration to a patient suffering from antigen-induced or exercise-induced asthma of a pharmaceutical composition

containing about 300 to about 2,000 units of heparin per kilogram of body weight in each dose, and preferably about 500 to about 1000 units/kg per dose.

As a general rule, heparin dosage is prescribed in units rather than milligrams. The U.S.P. standard for minimal heparin potency is 120 units/mg of dry material derived from lung tissue and 140 units/mg of dry material derived from other sources, with the U.S.P. unit being about 10% greater than the international unit (IU). The potency of commercial preparations ranges from 140 to 190 units/mg.

In accordance with the invention, a patient suffering from allergic or antigen-induced asthma is administered one dose of a heparin-containing inhalant composition from 1 to about 4 times daily, and preferably from 2 to 4 times daily, although more frequent administration can be utilized in the case of severe bronchospastic episodes. A patient suffering from exercise-induced asthma is administered one dose of a heparin-containing inhalant composition from about 30 minutes to about 3 hours before exercising. Additional doses may be given as needed during and after exercise.

Although it would be undesirable to administer heparin of significant anticoagulant potency to asthmatic patients in a manner which would cause the heparin to enter the bloodstream and induce hemorrhagic effects, it has been

discovered that even commercial heparin can be used in the method of the present invention without inducing such effects or increasing clotting time as measured by partial thromboplastin time (PTT). It is believed that the lack of anticoagulant activity observed with intrabronchially administered heparin results from the facts that (a) only a small amount, perhaps not more than 10%, of the active ingredient in a dose of any inhalant composition actually remains in the lungs and acts on lung tissue, and (b) heparin apparently binds to various sites in the bronchial mucosa and does not escape into the general circulation to any significant degree.

Nevertheless, it is preferred for purposes of the novel treatment methods to use as active ingredients low molecular weight heparins or heparin fragments having an average molecular weight of about 1,500 to about 6,000, and preferably from about 4000 to about 5000. Because of the reduced anticoagulant potency of the lower molecular weight heparin materials, there is little risk of adverse hemorrhagic effects associated with their usage; yet they exhibit excellent antiasthmatic properties.

Examples of low molecular weight heparin which may be used in the method and compositions of the present invention include: Fraxiparine (Choay, Paris, France), Lovenox (Pharmuka, Gennevilliers, France), Fragmin (Kabivitrum, Stockholm, Sweden), OP 2123 (Opocrin, Corlo,

Italy), RD heparin (Hepar, Franklin, Ohio), LHN-1 (Novo, Copenhagen, Denmark), CY222 (Choay) and unfractionated porcine mucosal heparins of sodium salt (Choay and Hepar). Although the in vivo potency of these various materials may differ significantly in terms of antithrombotic activity, Doutremepuich et al., Thromb.Res., 55: 419-426 (1989), they are all useful as antiasthmatic agents.

Natural and synthetic heparin fragments may also be used with great effectiveness in the subject method of treatment. Natural heparin fragments are those obtained by fractionation of commercial heparins by degree of affinity for antithrombin and subsequent extraction or chemical or enzymatic depolymerization to yield active and inactive fractions. Synthetic heparin fragments are sulfated oligosaccharides generally synthesized starting from glucose and glucosamine. Several examples of such fragments are set forth in Petitou, Nouv. Rev. Fr. Hematol., 26: 221-226 (1984), the disclosure of which is incorporated by reference herein.

Another form of heparin which is of particular value for use in the present method because of its almost total lack of anticoagulant activity is partially N-desulfated heparin. Whereas unfractionated heparin contains 100% N-sulfate groups, partially N-desulfated heparin preparations may have only 25-85% N-sulfate groups. Many heparins of this type have been found to have high

antithrombotic activity and low hemorrhagic effects.

Several examples of N-desulfated heparin preparations are disclosed in Sache et al., Thromb.Res., 55: 247-258 (1989) and Nagasawa et al., J. Biochem, 81: 989-993 (1977), the disclosures of which are incorporated by reference herein.

Any other form of heparin or heparin fragment which has little or no anticoagulant activity may also be used in the method of the present invention.

The term "heparin" as used hereinafter in unqualified form shall be understood as comprehending heparin (heparinic acid), commercial heparin, and those low molecular heparins, natural and synthetic heparin fragments or fractions, partially N-desulfated heparins and other non-anticoagulant heparins which exhibit anti-bronchospastic and anti-bronchoconstrictive activity.

The inhalant heparin compositions used in the present invention may comprise liquid or powdered compositions of heparin suitable for nebulization and intrabronchial use or aerosol compositions administered via an aerosol unit dispensing metered doses.

Suitable liquid compositions comprise heparin in an aqueous, pharmaceutically acceptable inhalant solvent, e.g., isotonic saline or bacteriostatic water. The solutions are administered by means of a pump or squeeze-actuated nebulized spray dispenser, or by any other conventional means for causing or enabling the requisite

dosage amount of the liquid composition to be inhaled into the patient's lungs.

Suitable powder compositions include, by way of illustration, powdered preparations of heparin thoroughly intermixed with lactose or other inert powders acceptable for intrabronchial administration. The powder composition can be administered via an aerosol dispenser or encased in a breakable capsule which may be inserted by the patient into a device that punctures the capsule and blows the powder out in a steady stream suitable for inhalation.

Aerosol formulations for use in the subject method typically include chlorofluorocarbon propellants, surfactants and co-solvents and may be filled into aluminum or other conventional aerosol containers which are then closed by a suitable metering valve and pressurized with propellant.

The concentration of heparin in any vehicle suitable for use in accordance with the present invention must be sufficiently high to provide the required dose of about 300-2,000 units of heparin/kg. Thus, for example, if a metered dose aerosol dispenser administers 4 ml of liquid per dose, the concentration of heparin in the aerosol in the case of a patient weighing 75 kg should be 5,625-37,500 units/ml.

As those skilled in the pharmaceutical arts will appreciate, many conventional methods and apparatus are

available for administering precisely metered doses of intrabronchial medicaments and for regulating the desired dosage amount in accordance with patient weight and severity of the patient's condition. Moreover, there are many art-recognized liquid, powdered and aerosol vehicles suitable for the intrabronchial heparin composition of the present invention. The invention is not limited to any particular inert vehicles, solvents or carriers and is not restricted to any particular methods or apparatus or intrabronchial administration.

The heparin compositions described herein provide highly effective and long-acting prophylaxis for antigen-induced and exercise-induced asthma. Many patients will require no more than two doses of intrabronchial heparin daily to remain symptom-free.

The following examples illustrate the methods and compositions of the invention and set forth various studies and experiments performed on animal and human subjects with respect thereto. These examples are not intended, however, to set forth compositions, procedures or dosage regimens which must be utilized exclusively to practice the invention.

EXAMPLE 1

Effect of inhaled heparin on antigen-induced bronchoconstriction. These experiments were conducted in 8

allergic sheep. Each animal was studied on 5 different experiment days, at least 2 weeks apart. For the control antigen experiment, after baseline measurements of specific lung resistance (SR_L) the sheep were challenged with aerosolized Ascaris suum antigen (400 breaths, 1:20 dilution), and measurements of SR_L repeated within 5 min. In order to evaluate the effect of aerosolized heparin on antigen-induced bronchoconstriction, on 3 separate days the sheep were pretreated with increasing doses of heparin (100, 300 and 1,000 units/kg), and antigen challenge was performed immediately thereafter. Measurements of SR_L were obtained before and after nebulization of heparin, and immediately after the antigen challenge (12).

On two separate occasions inhalation challenge with Ascaris suum antigen produced marked bronchoconstriction; mean \pm SE SR_L increased by $367 \pm 119\%$ and $314 \pm 88\%$ above baseline, respectively (fig. 1). Inhaled heparin per se had no effect on baseline SR_L , but it attenuated the antigen-induced bronchoconstrictor responses in a dose-dependent fashion. Mean SR_L increased by $313 \pm 87\%$, $151 \pm 69\%$ and $24 \pm 20\%$ above baseline with 100, 300 and 1,000 units/kg of heparin, respectively (fig. 1). The increases in SR_L with 300 and 1,000 units/kg of heparin were significantly lower than antigen control ($p < 0.05$).

EXAMPLE 2

Specificity of heparin action. - To study the specificity

of heparin action and to exclude the possibility that antiallergic action of heparin may be related to its ionic charge or alcohol preservative, additional experiments were conducted in allergic sheep on 3 separate days, and compared to control antigen data. Measurements of SR_L were obtained before and after the sheep were pretreated with 3 ml of either inhaled dextran sulfate (10mg/kg), benzyl alcohol preservative (0.01ml/ml) or De-N-sulfated heparin (10mg/kg). The sheep were then challenged with Ascaris suum antigen and measurements of SRL were repeated. The De-N-sulfated heparin dextran sulfate failed to attenuate the antigen-induced bronchoconstriction (fig. 2). These findings suggested that the inhibitory action of heparin is not related to its anionic molecular charge or mucopolysaccharide structure, as demonstrated by failure of dextran sulfate to modify antigen-induced bronchoconstriction. Failure of heparin diluent to modify antigen-induced airway responses excluded any non-specific effects of alcohol preservative; whereas failure of De-N-sulfated heparin also demonstrated the specificity of heparin action and underscored the importance of N-sulfated group of heparin molecule in the mediation of its antiallergic action.

EXAMPLE 3

Effect of heparin on compound 48/80-induced bronchoconstriction (n=7). - In order to test whether

heparin modifies both immunologic and non-immunologic mast cell-mediated reactions, additional experiments were done in sheep challenged with compound 48/80 which causes non-immunologic, non-cytolytic mast cell degranulation. These experiments were conducted on 2 separate days. After baseline measurements of SR_L the sheep received an inhalation challenge with compound 48/80 (400 breaths, 5% solution) and measurements of SR_L were repeated immediately thereafter. On a different experiment day, the sheep were pretreated with aerosolized heparin (1,000 units/kg); measurements of SR_L were obtained before and after heparin, and airway challenge with compound 48/80 was then performed, and measurements of SR_L repeated. Pretreatment with inhaled heparin (1,000 units/kg) markedly attenuated the compound 48/80-induced bronchoconstrictor responses; mean SR_L increased by $35 \pm 13\%$ above baseline, which was lower than the increase in SR_L of $374 \pm 72\%$ with compound 48/80 alone ($p < 0.05$) (fig.3).

EXAMPLE 4

Effect of heparin on agonist-induced bronchoconstriction.

- In order to exclude any direct effects of heparin on airway smooth muscle, we also studied the effect of inhaled heparin (1,000 units/kg) on carbachol (n=5) and histamine (n=8) induced bronchoconstriction. After obtaining baseline measurements of SR_L , the sheep were challenged with aerosolized carbachol (10 breaths, 2.5% solution) or

histamine (50 breaths, 5% solution) and measurements of SR_L were repeated. On separate days, the sheep were pretreated with inhaled heparin (1,000 units/kg) and the agonist challenges were repeated immediately thereafter, as described above.

The dose of inhaled heparin (1,000 units/kg) which markedly attenuated antigen- and compound 48/80-induced bronchoconstriction, failed to modify bronchoconstriction induced by carbachol and histamine. After carbachol challenge, mean SR_L increased by $652 \pm 73\%$ and $657 \pm 56\%$ above baseline, without and following pretreatment with heparin, respectively ($p=NS$). Aerosol histamine produced a similar degree of bronchoconstriction whether histamine was dissolved in buffered saline or in heparin solution ($\Delta SR_L = 258 \pm 49\%$ vs $175 \pm 40\%$) (fig. 3).

EXAMPLE 5

Effect of inhaled heparin on partial thromboplastin time.

In order to exclude any effect of inhaled heparin on PTT, venous blood (5 ml) was obtained before and 20 min after nebulization of heparin (1,000 units/kg) for analysis of PTT ($n=7$). The highest dose of inhaled heparin used in the study (1,000 units/kg) failed to modify the plasma partial thromboplastin time; mean values were 30 ± 2 and 31 ± 2 seconds before and after aerosol heparin ($p=NS$). In subsequent experiments, we have also observed that inhaled heparin (1,000 units/kg) in sheep does not alter PTT, when

studied immediately after nebulization, or 6-12 hours post-heparin. These findings suggested that the antiallergic action of heparin is probably related to non-anticoagulant properties of heparin.

EXAMPLE 6

Pharmacodynamics of heparin action. - In this

investigation, we studied the pharmacodynamics of antiallergic action of heparin. SR_L was measured in 8 sheep allergic to Ascaris suum antigen, before and after inhalation challenge with antigen. On 4 different days, antigen challenge was repeated after pretreatment with aerosol heparin (1,000 units/kg) administered 20 min, 6 hrs, 12 hrs and 24 hrs prior to antigen challenge. SR_L (mean \pm SE) increased by $374 \pm 116\%$ above baseline with antigen alone ($p < 0.05$). Aerosol heparin attenuated the antigen effects in a time-dependent fashion. The peak inhibitory effect of aerosol heparin was observed at 20 min pretreatment, and the degree of inhibition decreased with time; SR_L values were $31 \pm 29\%$, $99 \pm 38\%$, $142 \pm 40\%$ and $306 \pm 60\%$ for 20 min, 6 hrs, 12 hrs, and 24 hrs pretreatments, respectively (fig. 4). From these pharmacodynamic studies we concluded that antiallergic effects of heparin are time-related and the peak effect of inhaled heparin is observed soon after administration.

EXAMPLE 7

In vitro effects of heparin on antigen-induced trachealis muscle contraction. - We have also tested the antiallergic actions of heparin by determining if heparin blocked immunologically-induced tracheal smooth muscle contraction in vitro. Tracheal smooth muscle was obtained from sheep allergic to Ascaris suum antigen, and was suspended in an organ bath containing warmed (39°C) oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit buffer. Tissues were allowed to equilibrate for 1 hr at resting tension of 1 g. After the equilibration period, the tissues were treated with heparin (injection USP, Elkin-Sinn) at concentrations of 10, 100, or 1000 µg/ml (final concentration in the bath) dissolved in 10 µl DMSO. Two types of controls were used: vehicle (10 µl DMSO) treated tissues and tissues treated with the anti-asthma drug, nedocromil sodium (10⁻⁵M). Tissues were challenged, after a 30 min pretreatment, with 10, 30 and 100 µl of antigen (162,000 protein nitrogen units/ml). Contractions induced by antigen were expressed as a percentage of the contraction elicited by a maximally effective concentration of acetylcholine (100 mM). Antigen produced dose-dependent increases in tension, which were blocked by heparin and nedocromil sodium (fig. 5).

Neither heparin nor nedocromil sodium affect the maximum response to acetylcholine. Likewise, dose response curves to acetylcholine were unaffected by any

concentration of heparin used. The addition of the heparin preservative benzyl alcohol did not reverse acetylcholine-induced contractions or inhibit antigen-induced contractions in sheep tracheal smooth muscle, as has been observed in dog bronchi. These results support our in vivo findings and suggest that heparin blocks immunologically tracheal smooth muscle contraction without affecting agonist induced contraction. This action is similar to that of the anti-asthma drug nedocromil sodium and may be related to inhibition of mast cell mediator release.

EXAMPLE 8

Effect of heparin on exercise-induced bronchoconstriction

in patients with asthma (n=3). - Preliminary studies were

conducted in 3 subjects with history of marked exercise-

induced bronchoconstriction (EIB). These subjects were

studied on 3 different days, 7 days apart. On day 1, after

obtaining resting pulmonary function tests, the subjects

were screened for EIB. The subjects exercised on a

treadmill, with increasing speed and degree of inclination,

until their heart rate reached 85% of predicted maximum.

The achieved exercise work-load was then continued for 10

min. Throughout the study, the heart rate was monitored

continuously with an EKG. Minute ventilation, estimated

with a calibrated respiratory inductive plethysmograph,

measuring specific airway conductance (SG_{aw}) before,

immediately after the exercise, and serially every 5 min

for 30 min post-exercise.

After the initial screening day, the subjects were studied on 2 separate days, in a single-blind randomized fashion. The work-load estimated on the initial screening day was kept constant on the two test days. The subjects were pretreated with an aerosol (4ml) of either heparin (1,000 units/kg) or a placebo solution (0.01 ml/ml benzyl alcohol in bacteriostatic injection water). SG_{av} was obtained before and 45 min after nebulization of heparin or placebo solution. Exercise challenge was then performed, as stated above, and measurements of SG_{av} were obtained immediately after exercise and every 5 min for 30 min post-exercise. On both test days, the heart rate and minute ventilation were monitored as on a control day.

These studies in 3 subjects demonstrated that inhaled heparin (at a dose which blocked compound 48/80 and antigen-induced bronchoconstriction in sheep, i.e. 1,000 units/kg) markedly attenuated EIB (fig. 6). Both the magnitude as well as the duration of EIB were attenuated.

It has thus been shown that there are provided a method and compositions which achieve the various objects of the invention and which are will adapted to meet the conditions of practical use.

As various possible embodiments might be made of the above invention, and as various changes might be made

in the embodiments set forth above, it is to be understood that all matters herein described are to be interpreted as illustrative and not in a limiting sense.

What is claimed as new and desired to be protected by Letters Patent is set forth in the following claims.

I CLAIM:

1. A method of treating a patient suffering from antigen-induced or exercise-induced asthma comprising the intrabronchial administration to the patient of an inhalant composition containing about 300 to about 2,000 units of heparin per kilogram of patient body weight in each dose.

2. A method according to claim 1 wherein said patient is suffering from antigen-induced asthma.

3. A method according to claim 2 wherein said patient receives one to about four doses of said composition per day.

4. A method according to claim 3 wherein the patient receives from two to four doses of said composition per day.

5. A method according to claim 1 wherein said patient is suffering from exercise-induced asthma.

6. A method according to claim 5 wherein said patient receives one dose of said composition from about thirty minutes to about three hours before exercise.

7. A method according to claim 6 wherein said patient receives an additional dose of said composition during or after exercise.

8. A method according to claim 1 wherein said composition contains about 500 to about 1,000 units/kg of heparin per dose.

9. A method according to claim 8 wherein said composition contains about 1,000 units/kg of heparin per dose.

10. A method according to claim 1 wherein said heparin is commercial heparin.

11. A method according to claim 1 wherein said heparin is a low molecular weight heparin or heparin fragment having an average molecular weight of about 1,500 to about 6,000.

12. A method according to claim 11 wherein said low molecular weight heparin or heparin fragment has an average molecular weight of about 4,000 to about 5,000.

13. A method according to claim 1 wherein said heparin is a partially N-desulfated heparin or other non-anticoagulant heparin.

14. A method according to claim 13 wherein said partially N-desulfated heparin has from about 25 to about 85% of the N-sulfate groups of commercial heparin.

15. A method according to claim 1 wherein said inhalant composition is a solution of heparin in an aqueous, pharmaceutically acceptable inhalant vehicle.

16. A method according to claim 15 wherein said vehicle is isotonic saline or bacteriostatic water.

17. A method according to claim 15 wherein said composition includes about 5,625 to about 37,500 units of heparin per milliliter.

18. A method according to claim 15 wherein said composition is administered by means of a pump or squeeze-actuated nebulizer.

19. A method according to claim 15 wherein said composition further includes an aerosol propellant and is administered via a metered aerosol dose inhaler.

20. A method according to claim 1 wherein said inhalant composition comprises a powdered preparation of heparin intermixed with an inert powder acceptable for intrabronchial administration.

21. A method according to claim 20 wherein said inert powder is lactose.

22. A method according to claim 20 wherein said powder is administered via an aerosol dispenser.

23. A method according to claim 20 wherein said powder is administered from a breakable capsule.

24. A pharmaceutical composition for treatment of a patient suffering from antigen-induced or exercise-induced asthma comprising about 300 to about 2,000 units of heparin per kilogram of patient body weight in each dose in a pharmaceutically acceptable inhalant vehicle.

25. A composition according to claim 24 which comprises about 500 to about 1,000 units/kg of heparin per dose.

26. A composition according to claim 25 which comprises about 1,000 units/kg of heparin per dose.

27. A composition according to claim 24 wherein said heparin is commercial heparin.

28. A composition according to claim 24 wherein said heparin is a low molecular weight heparin or heparin fragment having an average molecular weight of about 1,500 to about 6,000.

29. A composition according to claim 28 wherein said low molecular weight heparin or heparin fragment has an average molecular weight of about 4,000 to about 5,000.

30. A composition according to claim 24 wherein said heparin is a partially N-desulfated heparin or other non-anticoagulant heparin.

31. A composition according to claim 30 wherein said partially N-desulfated heparin has from about 25 to about 85% of the N-sulfate groups of commercial heparin.

32. A composition according to claim 24 wherein said vehicle is an aqueous liquid.

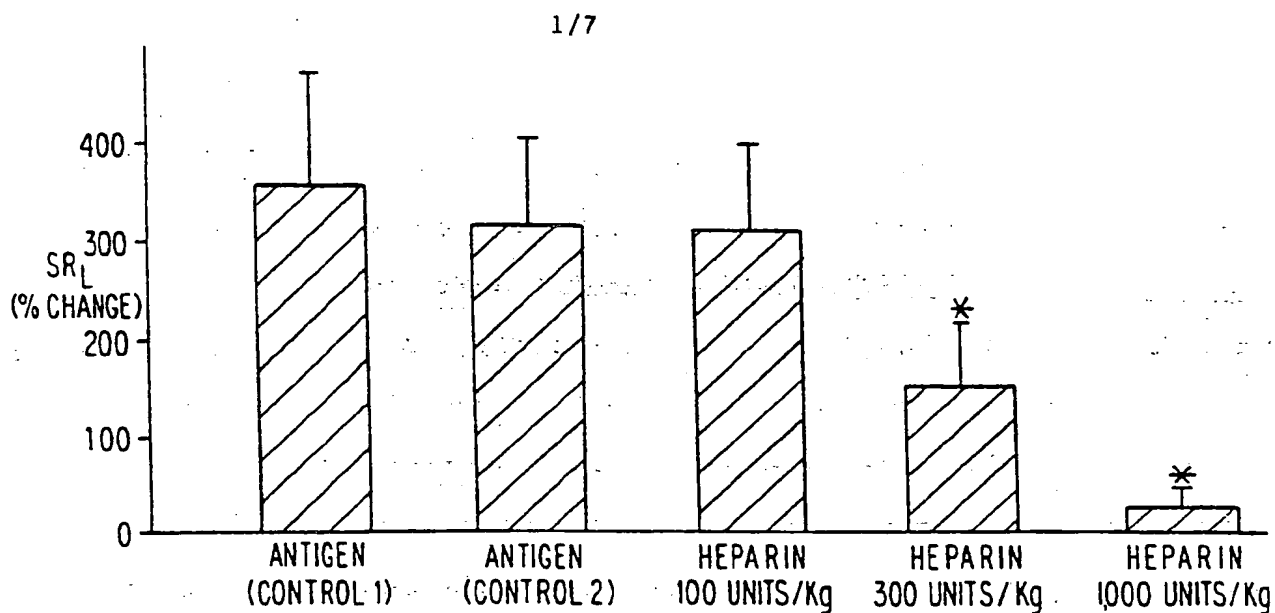
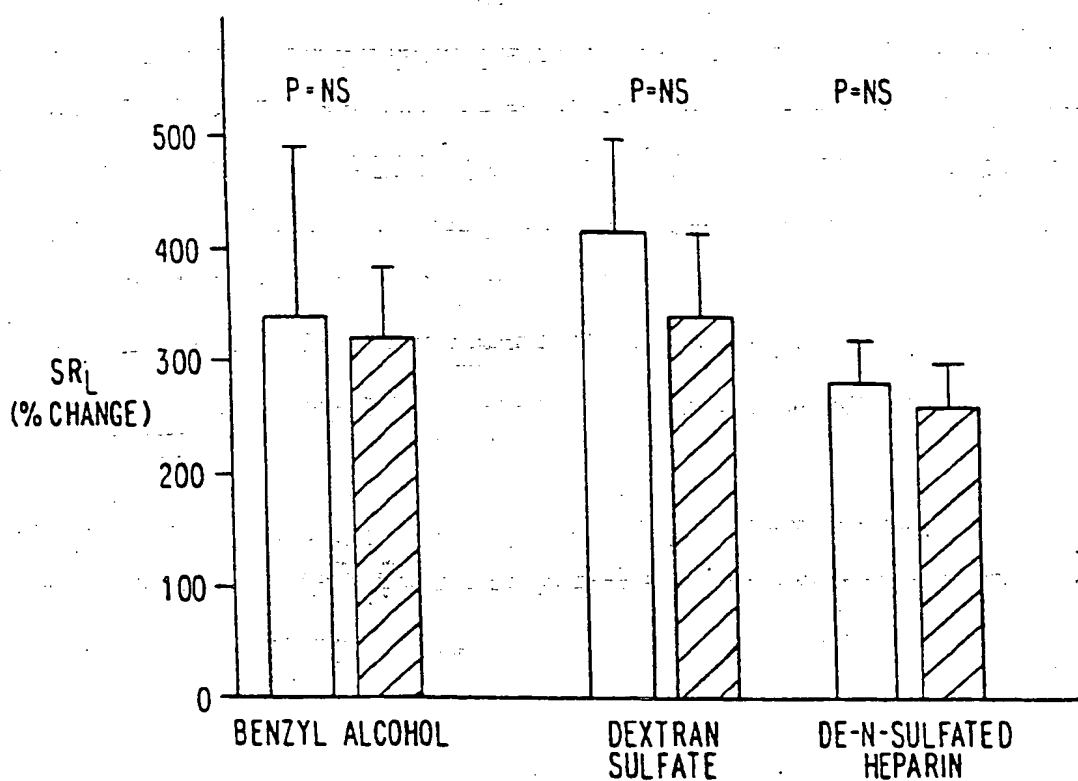
33. A composition according to claim 32 wherein said liquid is isotonic saline or bacteriostatic water.

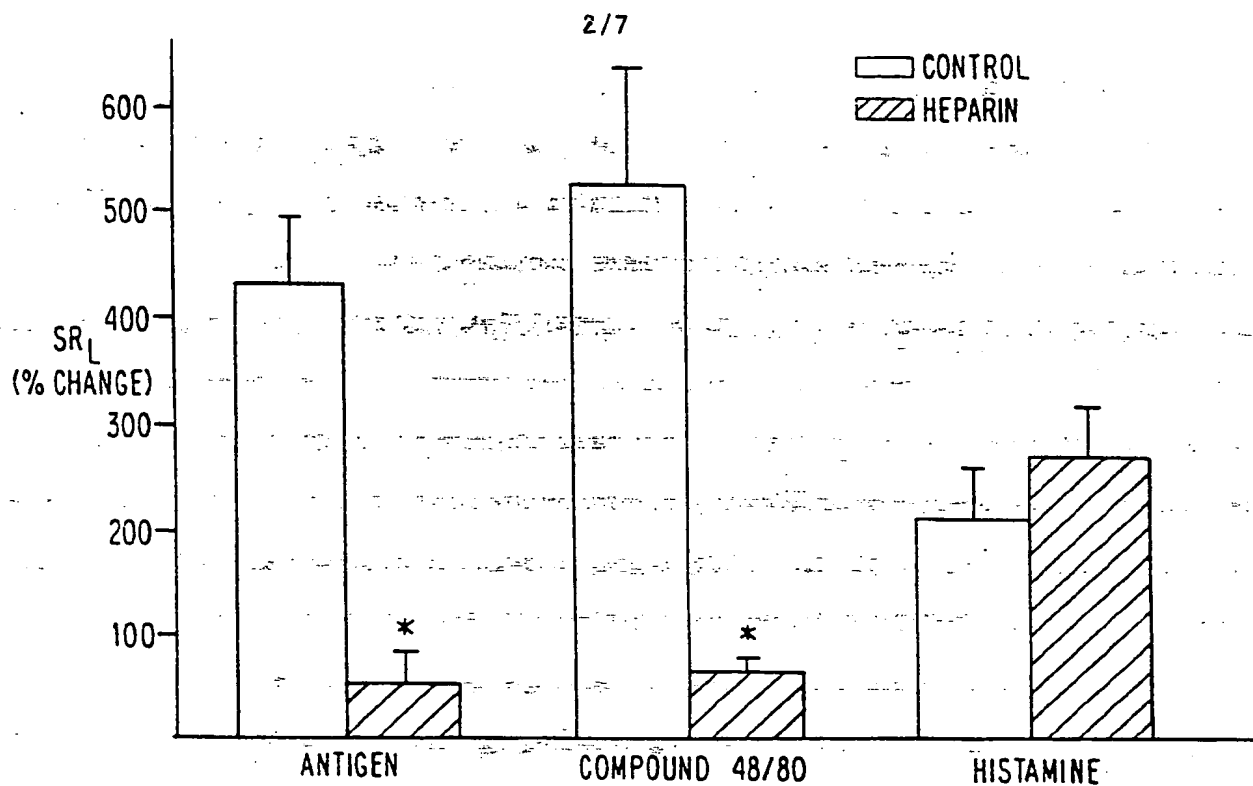
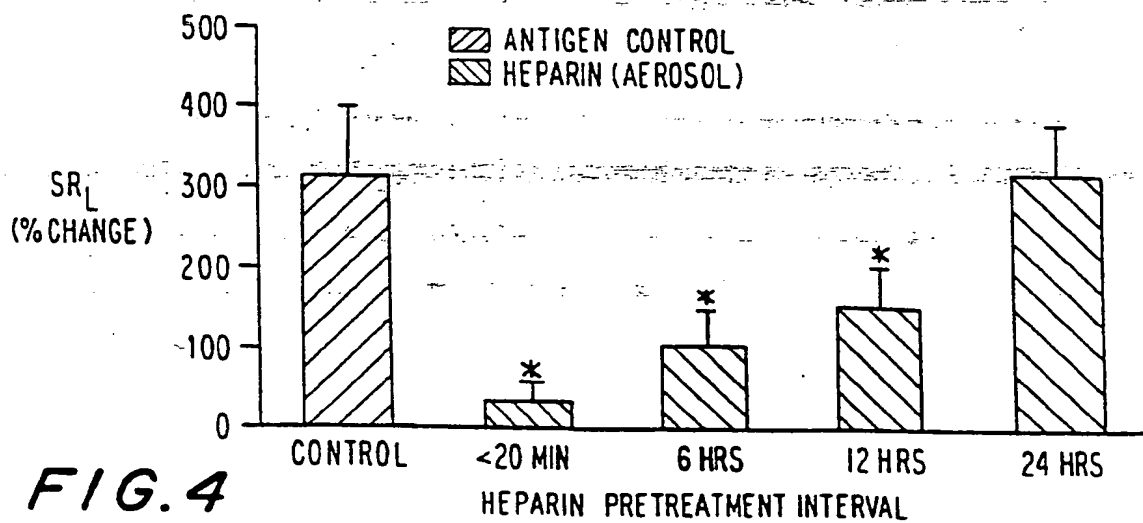
34. A composition according to claim 32 which includes about 5,625 to about 37,500 units of heparin per millimeter.

35. A composition according to claim 32 which additionally includes an aerosol propellant.

36. A composition according to claim 24 wherein said vehicle is a powder.

37. A composition according to claim 36 wherein said powder is lactose.

**FIG. 1****FIG. 2**

**FIG. 3****FIG. 4**

3/7

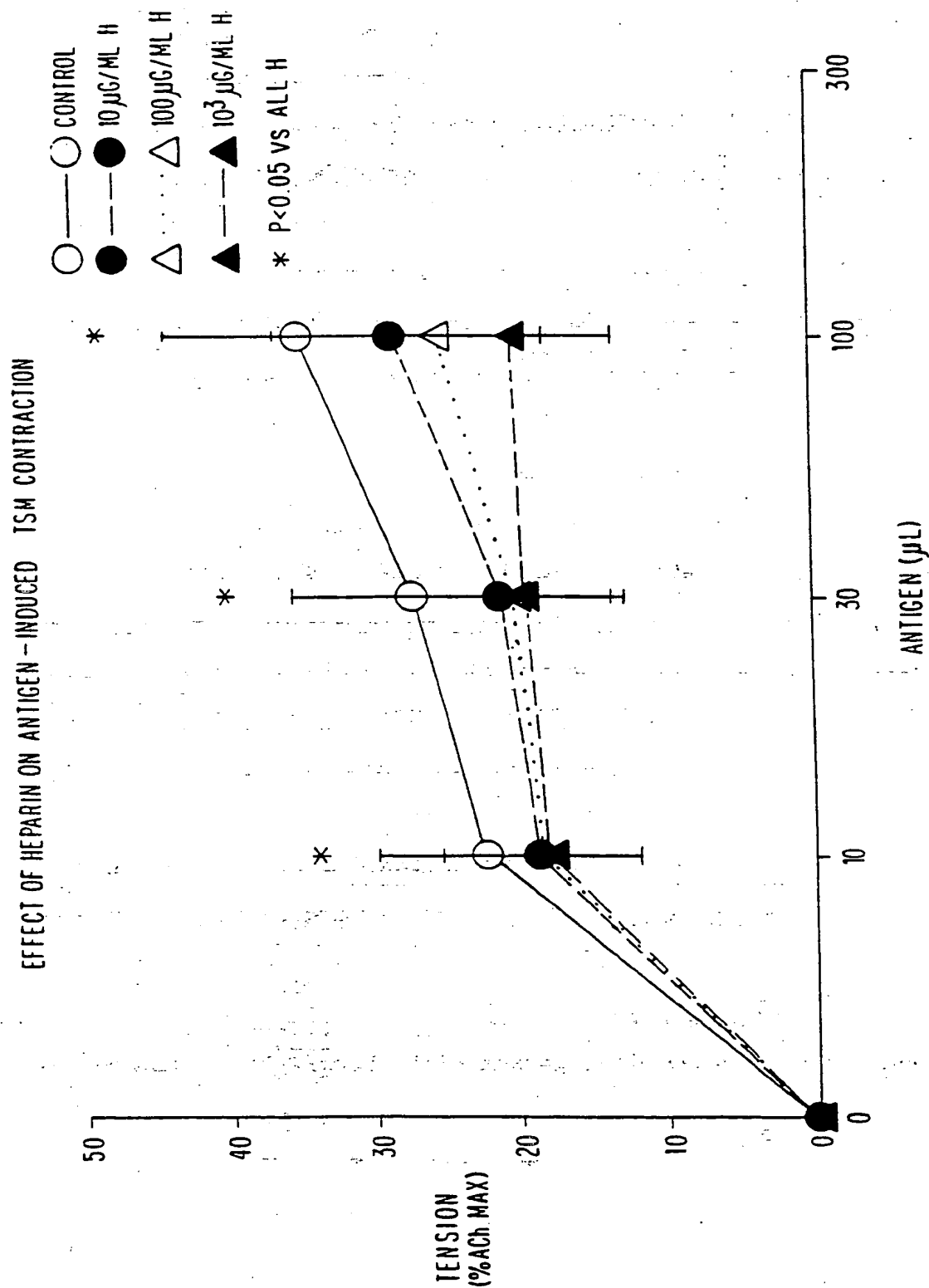


FIG. 5A

4/7

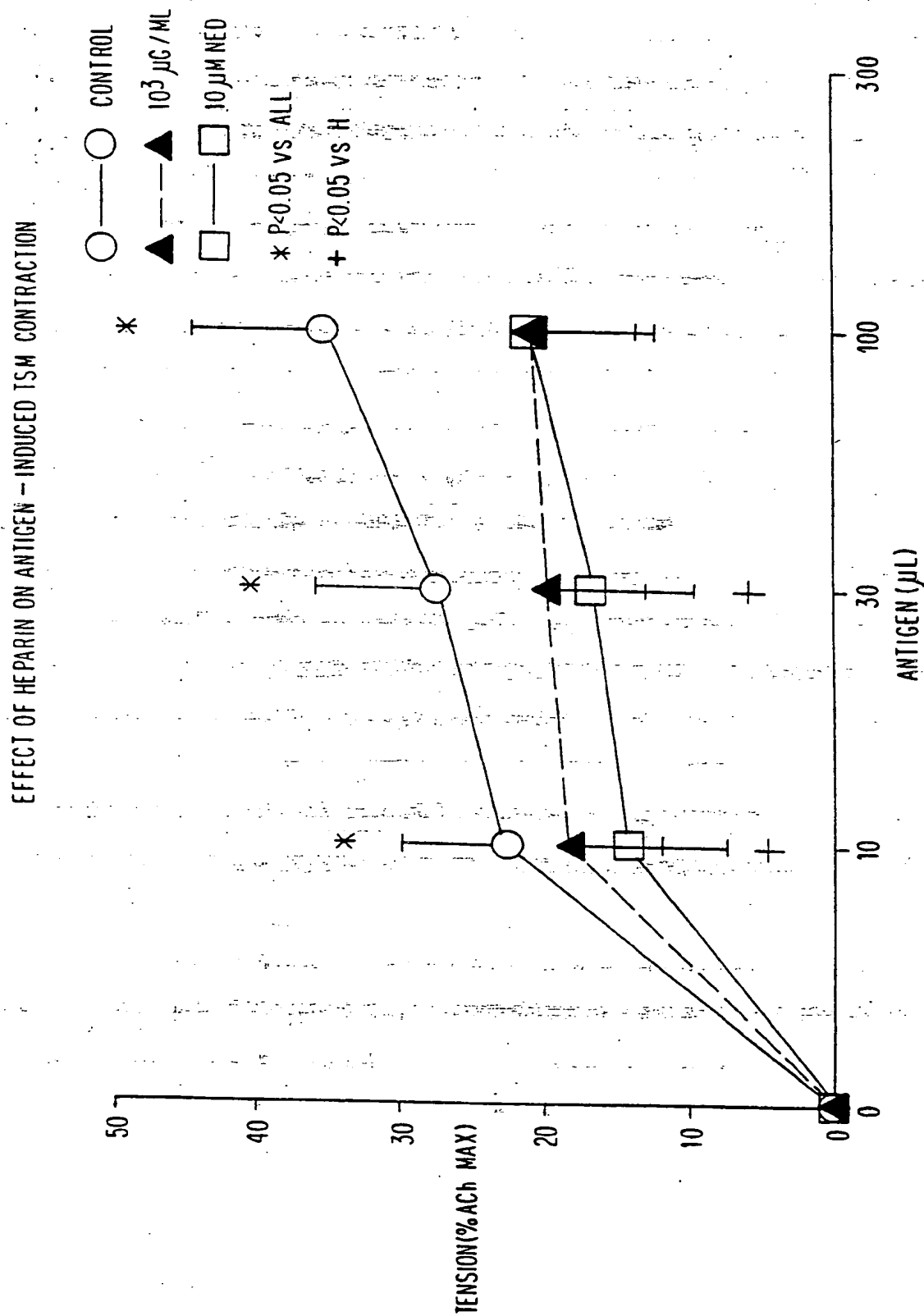
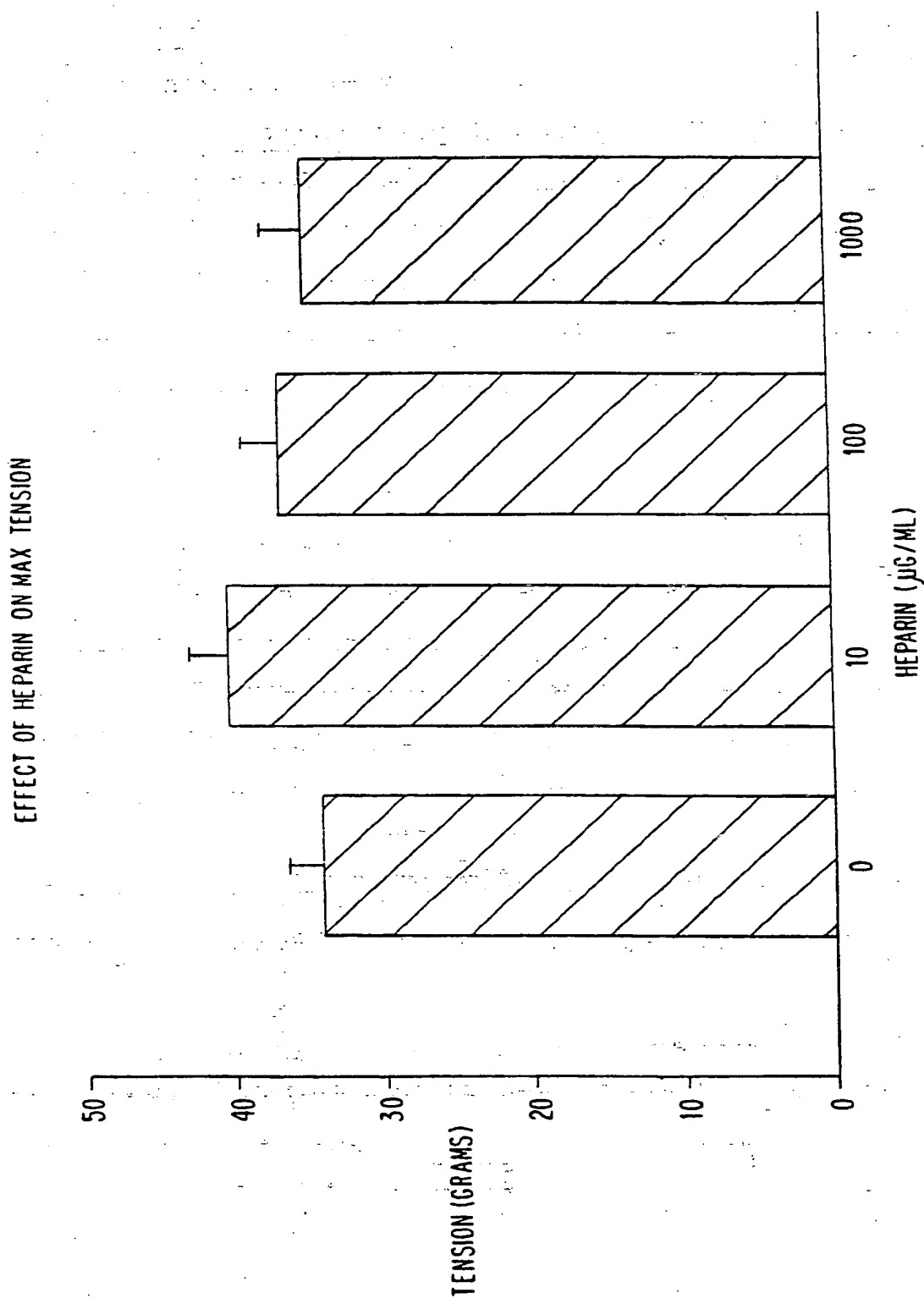
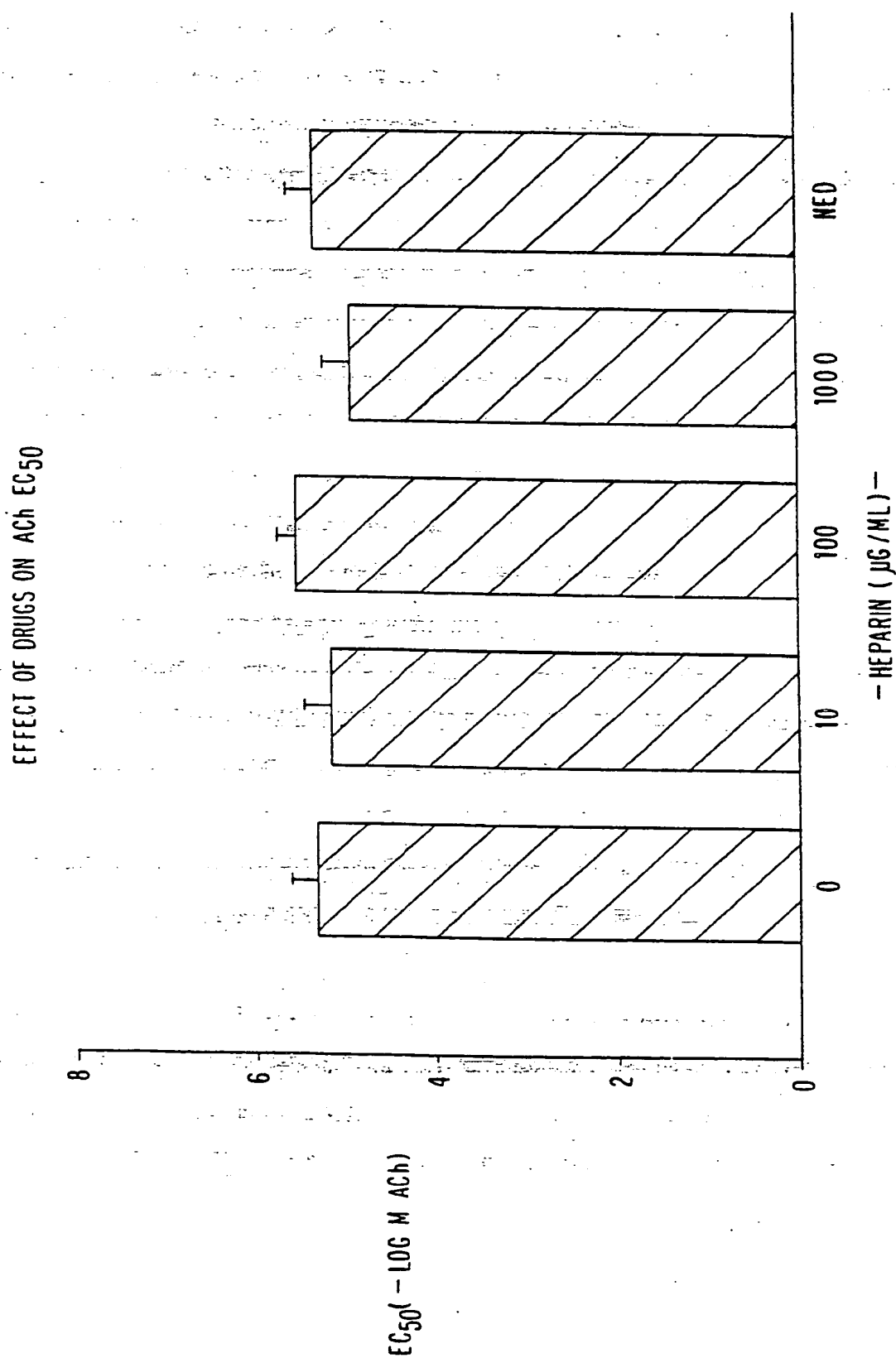


FIG. 5B

5/7

*FIG. 5C*

6/7

*FIG. 5D*

7/7

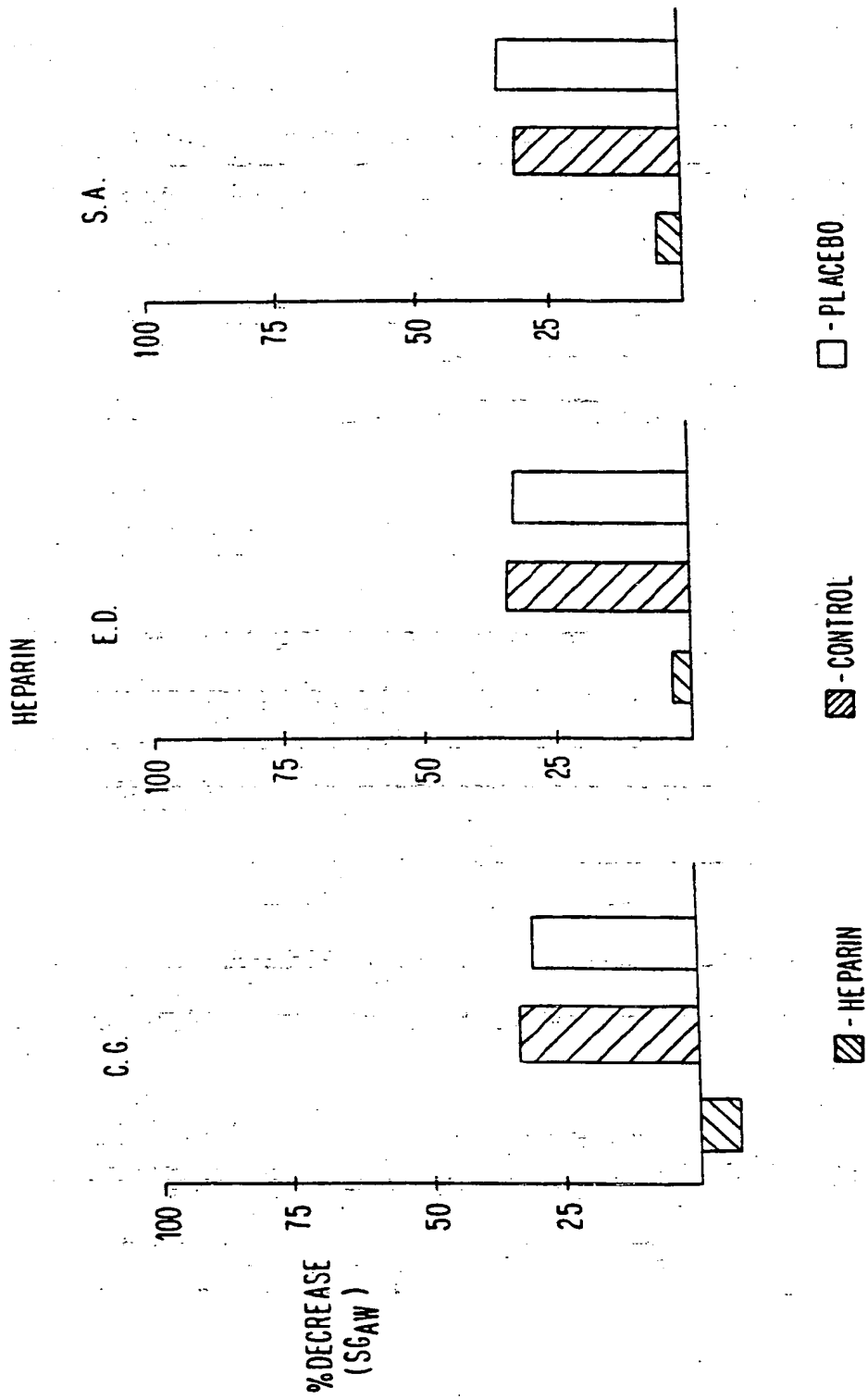


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/02880

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K-9/12, 31/725

US CL :514/54, 56; 536/21; 424/434, 488, 46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/54, 56; 536/21; 424/434, 488, 46

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online search terms: heparin, heparinoid, nasal?, inhalant, aerosol, nebulizer, asthma.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VUTR. BOLES, Vol. 26, no. 6, issued 1987, Petrov et al, "Inhalation treatment with low doses of heparin in bronchial asthma patients," abstract.	1-10, 15-16, 19-26, and 32-37
X	BIOLOGICAL ABSTRACTS, Vol. 85, no. 5, issued 01 March 1988, Ono et al, "Studies on Heparin in Allergic Reactions: 3. Therapeutic Use of Heparin in Bronchial Asthma," abstract no. 51021, Okayama Igakkai Zasshi, 99(5/6):559-568.	1-10, 15-16, 19-26, and 32-37
X	JP, A, 3-169821, (KISSEI PHARM KK) 23 July 1991, abstract.	1-37

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

A document defining the general state of the art which is not considered to be part of particular relevance	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
E earlier document published on or after the international filing date	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
O document referring to an oral disclosure, use, exhibition or other means	*Z* document member of the same patent family
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

06 May 1993

Date of mailing of the international search report

13 MAY 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Authorized officer

NANCY S. HUSARIK

Facsimile No. NOT APPLICABLE

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US93/02880

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, Vol. 113, no. 3, issued 16 July 1990, Landyshev et al, "Method of Treating Respiratory Insufficiency in Patients with Nonspecific Pulmonary Disease," see page 62, abstract no. 17897x.	24-27 and 32-37
X	Chemical Abstracts, Vol. 90, issued 1979, Moreau et al, "Pharmaceutical Based on Heparin for Tracheobronchial and Alveolar Administration," see page 312, abstract no. 43816w.	24-27 and 32-37
X	Chemical Abstracts, Vol. 102, issued 1985, Breitenstein et al, "Heparin Formulation Containing a Surfactant with Action on the Mucous Membranes of the Mouth, Nose, and/or the Throat," see page 352, abstract no. 154804b.	24-27 and 32-37
X	Chemical Abstracts, Vol. 108, issued 1988, Brown et al, "Dimethyl Sulfoxide with Heparin in the Treatment of Smoke Inhalation Injury," see page 69, abstract no. 198373p, J. Burn Care Rehabil. 9(1):22-5.	24-27 and 32-37
X	US, A, 5,037,810 (SALIBA, JR) 06 August 1991, see entire document.	24-27 and 32-37
X	Chemical Abstracts, Vol. 109, issued 1988, Johansen et al, "Nasal Pharmaceuticals Containing Low-Molecular Weight Heparin and a Fusidate," see page 370, abstract no. 116061u.	28-31
X	US, A, 5,032,679 (BRANDLEY ET AL) 16 July 1991, see entire document.	1, 11-14, and 28-31



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification ⁵ : A61K 9/12, 31/725</p>	<p>A1</p>	<p>(11) International Publication Number: WO 93/19734 (43) International Publication Date: 14 October 1993 (14.10.93)</p>
<p>(21) International Application Number: PCT/US93/02880 (22) International Filing Date: 26 March 1993 (26.03.93) (30) Priority data: 07/862,448 2 April 1992 (02.04.92) US (71) Applicant: BAKER NORTON PHARMACEUTICALS, INC. [US/US]; 8800 N.W. 36th Street, Miami, FL 33178 (US). (72) Inventor: AHMED, Tahir ; 3705 Granada Boulevard, Coral Gables, FL 33134 (US). (74) Agent: SCHIFFMILLER, Martin, W.; Kirschstein, Ottinger, Israel & Schiffmiller, 551 Fifth Avenue, New York, NY 10176-0024 (US).</p>		<p>(81) Designated States: AU, CA, FI, JP, KR, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i></p>
<p>(54) Title: METHOD AND COMPOSITION FOR TREATING ANTIGEN-INDUCED AND EXERCISE-INDUCED ASTHMA</p> <p>(57) Abstract</p> <p>A method of treating a patient suffering from antigen-induced or exercise-induced asthma comprising the intrabronchial administration to the patient of an inhalant composition containing about 300 to about 2,000 units of heparin per kilogram of patient body weight in each dose. Commercial heparin, low weight heparins or heparin fragments and partially N-desulfated heparins or other non-anticoagulant heparins may be used. Suitable inhalant compositions for use in the novel treatment method are also provided.</p>		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCI on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	FR	France	MR	Mauritania
AU	Australia	GA	Gabon	MW	Malawi
BB	Barbados	GB	United Kingdom	NL	Netherlands
BE	Belgium	GN	Guinea	NO	Norway
BF	Burkina Faso	GR	Greece	NZ	New Zealand
BG	Bulgaria	HU	Hungary	PL	Poland
BJ	Benin	IE	Ireland	PT	Portugal
BR	Brazil	IT	Italy	RO	Romania
CA	Canada	JP	Japan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CC	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SK	Slovak Republic
CI	Côte d'Ivoire	LI	Liechtenstein	SN	Senegal
CM	Cameroon	LK	Sri Lanka	SU	Soviet Union
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	MC	Monaco	TG	Togo
DE	Germany	MG	Madagascar	UA	Ukraine
DK	Denmark	ML	Mali	US	United States of America
ES	Spain	MN	Mongolia	VN	Viet Nam
FI	Finland				

**METHOD AND COMPOSITION FOR TREATING
ANTIGEN-INDUCED AND EXERCISE-INDUCED ASTHMA**

BACKGROUND OF THE INVENTION

1. Field of the Invention

The invention relates to methods of preventing and reversing the symptoms and manifestations of asthma.

2. Description of the Prior Art

Chronic asthma can be considered to be predominantly an inflammatory disease with associated bronchospasm. The degree of reactivity and narrowing of the bronchi in response to stimuli is greater in asthmatics than in normal individuals. Persistent inflammation is responsible for the bronchial hyperreactivity. Mucosal edema and mucus plugging and hypersecretion may be present; pulmonary parenchyma is normal. Airway narrowing may reverse spontaneously or with therapy. Type 1 (immediate) immune responses may play an important role in the development of asthma in children and many adults; however, when onset of disease occurs in adulthood, allergic factors may be difficult to identify. Exposure to cold dry air, exercise and other aggravating factors also may trigger asthma.

The most common symptoms of asthma are breathlessness and chest tightness; wheezing, dyspnea, and cough also are prominent. Reduced pulmonary function typical of obstructive rather than restrictive airway

disease is usually observed. Asymptomatic periods often alternate with paroxysms.

Of the known triggers of asthma, allergens and exercise have received the most attention. Both are powerful, naturally occurring stimuli; exercise is a potential factor in the daily life of every asthmatic, whereas allergens only effect some persons with asthma. Nevertheless, more is known about the effects of antigen, and it is only in the last several years that interest in the pathophysiology and treatment of exercise-induced bronchoconstriction (EIB) has developed. The bulk of current evidence demonstrates that the essential stimulus for the development of the bronchoconstriction is a fall in the temperature of the airways secondary to heat and water loss, that occur during the conditioning of inspired air. If heat loss and airway cooling are prevented, obstruction to airflow does not develop, and if they are augmented by lowering the inspired air temperature and/or water content, or by increasing the level of ventilation, the obstruction proportionately worsens.

Despite these developments, it is not yet known how airway cooling produces its effects. There are a series of observations in the literature which suggest that mediators of immediate hypersensitivity may play a role. Although the direct effect of physical stimuli like cold on primed asthmatic airway smooth muscle has been proposed by

some, others believe that these physical stimuli are capable of degranulating mast cells, thus suggesting a central role of mast cell mediators in EIB. This however remains controversial. Recently, it has been demonstrated that leukotriene antagonists may attenuate EIB, thus underscoring the importance of leukotrienes and not histamine release from mast cells during EIB.

The general goals of drug therapy for asthma are prevention of bronchospasm and long-term control of bronchial hyperreactivity. Because it is usually not possible for either patient or physician to predict when bronchospasm may occur, patients with all but the most episodic and/or entirely seasonal attacks may require continuous therapy.

Beta agonists are useful; they stimulate beta₂-adrenergic receptors, increase intracellular cAMP, and inhibit the release of inflammatory mediators. Other useful drugs include theophylline and related xanthine drugs, which produce bronchodilation through unknown mechanisms; the bismolone, cromolyn, which prevents the release of mediator substances and blocks respiratory neuronal reflexes, and corticosteroids, which primarily decrease inflammation and edema. Anticholinergic drugs may relieve bronchospasm by blocking parasympathetic cholinergic impulses at the receptor level. Antihistamines occasionally prevent or abort allergic asthmatic episodes,

particularly in children, but they can only be partially effective in asthma because histamine is only one of many mediators.

Standard treatment for exercise-induced asthma includes the use of an inhalant. Beta₂-adrenergic agents administered 15 minutes before exercise provide some protection from broncho-constriction for up to four hours, and cromolyn sodium administered up to two hours prior to exercise may afford limited protection in some patients.

The current drug modalities used for treatment of allergy-induced and exercise-induced asthma suffer from a number of drawbacks. In general, the conventional agents have a relatively short duration of action and must be repeatedly administered for prophylaxis. Moreover, because of serious adverse effects associated with the use of agents such as beta₂-adrenergic agonists and corticosteroids, the therapeutic margin of safety with such agents is relatively narrow and patients using them must be carefully monitored. In the case of exercise-induced asthma or bronchoconstriction, the current treatment regimens are inadequate and often of only limited prophylactic value.

SUMMARY OF THE INVENTION

It is an object of the present invention to

provide a method and compositions for treatment of both antigen-induced and exercise-induced asthma which do not suffer from the drawbacks of the prior art.

It is a further object of the present invention to provide a method and compositions for the treatment of asthma which have long-term prophylactic effects and are also effective in reversing the manifestations of an asthmatic episode.

Still another object of the present invention is to provide a method and compositions as described above which are highly effective in preventing the onset of exercise-induced asthma.

Yet a further object of the present invention is to provide a method and compositions as described above which are safe, simple and relatively inexpensive.

In keeping with these objects and others which will become apparent hereinafter, the invention resides in a method of treating a patient suffering from antigen-induced or exercise-induced asthma through the intrabronchial administration to the patient of a pharmaceutical composition comprising from about 300 to about 2,000 units of heparin per kilogram of patient body weight in each dose, with about 1 to 4 doses administered daily. Novel inhalant heparin compositions are also provided in the form of liquid or powdered nebulizer or aerosol compositions containing suitable concentrations of

heparin.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a bar graph showing the effect of increasing doses of inhaled heparin on bronchoconstriction, with data given as mean \pm SE \pm increase SR_L (specific lung resistance).

FIG. 2 is a bar graph comparing the effects of benzyl alcohol, dextran sulfate and N-desulfated heparin on antigen-induced bronchoconstriction. The open bars represent control data, while post-drug treatment data are shown in hatched bars.

FIG. 3 is a bar graph comparing the effects of inhaled heparin on bronchoconstriction responses induced by antigen, compound 48/80 and histamine.

FIG. 4 is a bar graph showing the time-dependent inhibition of antigen-induced bronchoconstriction by inhaled heparin (1,000 units/kg). Data are shown as mean \pm SE. Starred values are significantly different from control ($p < 0.05$).

FIG. 5 includes four graphs showing the in vitro effects of heparin on antigen-induced tracheal smooth muscle contraction in sheep:

5A - Shows the inhibition of antigen-induced contraction by increasing doses of heparin; the tension is plotted as a percent of the acetylcholine maximum reflected

in 5C.

5B - Shows comparative inhibition by heparin (H) and nedocromil (NED) of antigen-induced tracheal muscle contraction.

5C - Shows failure of heparin to modify acetylcholine-maximal tension.

5D - Shows failure of heparin and nedocromil to modify acetylcholine-tracheal muscle contraction as shown by EC_{50} .

FIG. 6 includes bar graphs reflecting the effects of inhaled heparin (1,000 units/kg) on exercise-induced bronchoconstriction in 3 human subjects, with data shown as percent decrease in specific airway conductance (SG_{aw}).

DETAILED DESCRIPTION OF THE INVENTION

Heparin, a sulfated mucopolysaccharide, is synthesized in mast cells as a proteoglycan and is particularly abundant in the lungs of various animals. Heparin is not a specific compound of fixed molecular weight but is actually a heterogenous mixture of variably sulfated polysaccharide chains composed of repeating units of D-glucosamine and either L-iduronic or D-glucuronic acids. The average molecular weight of heparin isolated from animal tissues ranges from about 6,000 to about 30,000 Da.

Pharmacologically, heparin is known primarily as an anticoagulant. This activity results from heparin's

ability to bind to some of the residues of antithrombin III (AT-III), accelerating the neutralization by AT-III of activated clotting factors and preventing the conversion of prothrombin to thrombin. Larger amounts of heparin can inactivate thrombin and earlier clotting factors, preventing conversion of fibrinogen to fibrin.

The anticoagulant activity of heparin is related to the molecular weight of its polysaccharide fragments; low molecular weight components or fragments (for example, fragments having a molecular weight of less than 6,000) have moderate to low antithrombin and hemorrhagic effects. Similarly, low molecular weight heparins isolated from animal tissue have reduced anticoagulant properties because they consist primarily of the lower molecular weight fragments or fractions.

Commercial heparin, which is generally derived from beef lung or pork intestinal mucosa, has an average molecular weight of about 15,000-17,500.

It has now been discovered, surprisingly, that heparin administered intrabronchially is a potent and long-acting inhibitor of bronchospasm and bronchoconstriction and is consequently of great value in preventing and treating asthmatic episodes. Accordingly, the method of the present invention comprises the intrabronchial administration to a patient suffering from antigen-induced or exercise-induced asthma of a pharmaceutical composition

containing about 300 to about 2,000 units of heparin per kilogram of body weight in each dose, and preferably about 500 to about 1000 units/kg per dose.

As a general rule, heparin dosage is prescribed in units rather than milligrams. The U.S.P. standard for minimal heparin potency is 120 units/mg of dry material derived from lung tissue and 140 units/mg of dry material derived from other sources, with the U.S.P. unit being about 10% greater than the international unit (IU). The potency of commercial preparations ranges from 140 to 190 units/mg.

In accordance with the invention, a patient suffering from allergic or antigen-induced asthma is administered one dose of a heparin-containing inhalant composition from 1 to about 4 times daily, and preferably from 2 to 4 times daily, although more frequent administration can be utilized in the case of severe bronchospastic episodes. A patient suffering from exercise-induced asthma is administered one dose of a heparin-containing inhalant composition from about 30 minutes to about 3 hours before exercising. Additional doses may be given as needed during and after exercise.

Although it would be undesirable to administer heparin of significant anticoagulant potency to asthmatic patients in a manner which would cause the heparin to enter the bloodstream and induce hemorrhagic effects, it has been

discovered that even commercial heparin can be used in the method of the present invention without inducing such effects or increasing clotting time as measured by partial thromboplastin time (PTT). It is believed that the lack of anticoagulant activity observed with intrabronchially administered heparin results from the facts that (a) only a small amount, perhaps not more than 10%, of the active ingredient in a dose of any inhalant composition actually remains in the lungs and acts on lung tissue, and (b) heparin apparently binds to various sites in the bronchial mucosa and does not escape into the general circulation to any significant degree.

Nevertheless, it is preferred for purposes of the novel treatment methods to use as active ingredients low molecular weight heparins or heparin fragments having an average molecular weight of about 1,500 to about 6,000, and preferably from about 4000 to about 5000. Because of the reduced anticoagulant potency of the lower molecular weight heparin materials, there is little risk of adverse hemorrhagic effects associated with their usage; yet they exhibit excellent antiasthmatic properties.

Examples of low molecular weight heparin which may be used in the method and compositions of the present invention include: Fraxiparine (Choay, Paris, France), Lovenox (Pharmuka, Gennevilliers, France), Fragmin (Kabivitrum, Stockholm, Sweden), OP 2123 (Opocrin, Corlo,

Italy), RD heparin (Hepar, Franklin, Ohio), LHN-1 (Novo, Copenhagen, Denmark), CY222 (Choay) and unfractionated porcine mucosal heparins of sodium salt (Choay and Hepar). Although the in vivo potency of these various materials may differ significantly in terms of antithrombotic activity, Doutremepuich et al., Thromb.Res., 55: 419-426 (1989), they are all useful as antiasthmatic agents.

Natural and synthetic heparin fragments may also be used with great effectiveness in the subject method of treatment. Natural heparin fragments are those obtained by fractionation of commercial heparins by degree of affinity for antithrombin and subsequent extraction or chemical or enzymatic depolymerization to yield active and inactive fractions. Synthetic heparin fragments are sulfated oligosaccharides generally synthesized starting from glucose and glucosamine. Several examples of such fragments are set forth in Petitou, Nouv. Rev. Fr. Hematol., 26: 221-226 (1984), the disclosure of which is incorporated by reference herein.

Another form of heparin which is of particular value for use in the present method because of its almost total lack of anticoagulant activity is partially N-desulfated heparin. Whereas unfractionated heparin contains 100% N-sulfate groups, partially N-desulfated heparin preparations may have only 25-85% N-sulfate groups. Many heparins of this type have been found to have high

antithrombotic activity and low hemorrhagic effects.

Several examples of N-desulfated heparin preparations are disclosed in Sache et al., Thromb.Res., 55: 247-258 (1989) and Nagasawa et al., J. Biochem, 81: 989-993 (1977), the disclosures of which are incorporated by reference herein.

Any other form of heparin or heparin fragment which has little or no anticoagulant activity may also be used in the method of the present invention.

The term "heparin" as used hereinafter in unqualified form shall be understood as comprehending heparin (heparinic acid), commercial heparin, and those low molecular heparins, natural and synthetic heparin fragments or fractions, partially N-desulfated heparins and other non-anticoagulant heparins which exhibit anti-bronchospastic and anti-bronchoconstrictive activity.

The inhalant heparin compositions used in the present invention may comprise liquid or powdered compositions of heparin suitable for nebulization and intrabronchial use or aerosol compositions administered via an aerosol unit dispensing metered doses.

Suitable liquid compositions comprise heparin in an aqueous, pharmaceutically acceptable inhalant solvent, e.g., isotonic saline or bacteriostatic water. The solutions are administered by means of a pump or squeeze-actuated nebulized spray dispenser, or by any other conventional means for causing or enabling the requisite

dosage amount of the liquid composition to be inhaled into the patient's lungs.

Suitable powder compositions include, by way of illustration, powdered preparations of heparin thoroughly intermixed with lactose or other inert powders acceptable for intrabronchial administration. The powder composition can be administered via an aerosol dispenser or encased in a breakable capsule which may be inserted by the patient into a device that punctures the capsule and blows the powder out in a steady stream suitable for inhalation.

Aerosol formulations for use in the subject method typically include chlorofluorocarbon propellants, surfactants and co-solvents and may be filled into aluminum or other conventional aerosol containers which are then closed by a suitable metering valve and pressurized with propellant.

The concentration of heparin in any vehicle suitable for use in accordance with the present invention must be sufficiently high to provide the required dose of about 300-2,000 units of heparin/kg. Thus, for example, if a metered dose aerosol dispenser administers 4 ml of liquid per dose, the concentration of heparin in the aerosol in the case of a patient weighing 75 kg should be 5,625-37,500 units/ml.

As those skilled in the pharmaceutical arts will appreciate, many conventional methods and apparatus are

available for administering precisely metered doses of intrabronchial medicaments and for regulating the desired dosage amount in accordance with patient weight and severity of the patient's condition. Moreover, there are many art-recognized liquid, powdered and aerosol vehicles suitable for the intrabronchial heparin composition of the present invention. The invention is not limited to any particular inert vehicles, solvents or carriers and is not restricted to any particular methods or apparatus or intrabronchial administration.

The heparin compositions described herein provide highly effective and long-acting prophylaxis for antigen-induced and exercise-induced asthma. Many patients will require no more than two doses of intrabronchial heparin daily to remain symptom-free.

The following examples illustrate the methods and compositions of the invention and set forth various studies and experiments performed on animal and human subjects with respect thereto. These examples are not intended, however, to set forth compositions, procedures or dosage regimens which must be utilized exclusively to practice the invention.

EXAMPLE 1

Effect of inhaled heparin on antigen-induced bronchoconstriction. These experiments were conducted in 8

allergic sheep. Each animal was studied on 5 different experiment days, at least 2 weeks apart. For the control antigen experiment, after baseline measurements of specific lung resistance (SR_L) the sheep were challenged with aerosolized Ascaris suum antigen (400 breaths, 1:20 dilution), and measurements of SR_L repeated within 5 min. In order to evaluate the effect of aerosolized heparin on antigen-induced bronchoconstriction, on 3 separate days the sheep were pretreated with increasing doses of heparin (100, 300 and 1,000 units/kg), and antigen challenge was performed immediately thereafter. Measurements of SR_L were obtained before and after nebulization of heparin, and immediately after the antigen challenge (12).

On two separate occasions inhalation challenge with Ascaris suum antigen produced marked bronchoconstriction; mean \pm SE SR_L increased by $367 \pm 119\%$ and $314 \pm 88\%$ above baseline, respectively (fig. 1). Inhaled heparin per se had no effect on baseline SR_L , but it attenuated the antigen-induced bronchoconstrictor responses in a dose-dependent fashion. Mean SR_L increased by $313 \pm 87\%$, $151 \pm 69\%$ and $24 \pm 20\%$ above baseline with 100, 300 and 1,000 units/kg of heparin, respectively (fig. 1). The increases in SR_L with 300 and 1,000 units/kg of heparin were significantly lower than antigen control ($p < 0.05$).

EXAMPLE 2

Specificity of heparin action. - To study the specificity

of heparin action and to exclude the possibility that antiallergic action of heparin may be related to its ionic charge or alcohol preservative, additional experiments were conducted in allergic sheep on 3 separate days, and compared to control antigen data. Measurements of SR_L were obtained before and after the sheep were pretreated with 3 ml of either inhaled dextran sulfate (10mg/kg), benzyl alcohol preservative (0.01ml/ml) or De-N-sulfated heparin (10mg/kg). The sheep were then challenged with Ascaris suum antigen and measurements of SRL were repeated. The De-N-sulfated heparin dextran sulfate failed to attenuate the antigen-induced bronchoconstriction (fig. 2). These findings suggested that the inhibitory action of heparin is not related to its anionic molecular charge or mucopolysaccharide structure, as demonstrated by failure of dextran sulfate to modify antigen-induced bronchoconstriction. Failure of heparin diluent to modify antigen-induced airway responses excluded any non-specific effects of alcohol preservative; whereas failure of De-N-sulfated heparin also demonstrated the specificity of heparin action and underscored the importance of N-sulfated group of heparin molecule in the mediation of its antiallergic action.

EXAMPLE 3

Effect of heparin on compound 48/80-induced bronchoconstriction (n=7). - In order to test whether

heparin modifies both immunologic and non-immunologic mast cell-mediated reactions, additional experiments were done in sheep challenged with compound 48/80 which causes non-immunologic, non-cytolytic mast cell degranulation. These experiments were conducted on 2 separate days. After baseline measurements of SR_L the sheep received an inhalation challenge with compound 48/80 (400 breaths, 5% solution) and measurements of SR_L were repeated immediately thereafter. On a different experiment day, the sheep were pretreated with aerosolized heparin (1,000 units/kg); measurements of SR_L were obtained before and after heparin, and airway challenge with compound 48/80 was then performed, and measurements of SR_L repeated. Pretreatment with inhaled heparin (1,000 units/kg) markedly attenuated the compound 48/80-induced bronchoconstrictor responses; mean SR_L increased by $35 \pm 13\%$ above baseline, which was lower than the increase in SR_L of $374 \pm 72\%$ with compound 48/80 alone ($p < 0.05$) (fig.3).

EXAMPLE 4

Effect of heparin on agonist-induced bronchoconstriction.

- In order to exclude any direct effects of heparin on airway smooth muscle, we also studied the effect of inhaled heparin (1,000 units/kg) on carbachol (n=5) and histamine (n=8) induced bronchoconstriction. After obtaining baseline measurements of SR_L , the sheep were challenged with aerosolized carbachol (10 breaths, 2.5% solution) or

histamine (50 breaths, 5% solution) and measurements of SR_L were repeated. On separate days, the sheep were pretreated with inhaled heparin (1,000 units/kg) and the agonist challenges were repeated immediately thereafter, as described above.

The dose of inhaled heparin (1,000 units/kg) which markedly attenuated antigen- and compound 48/80-induced bronchoconstriction, failed to modify bronchoconstriction induced by carbachol and histamine. After carbachol challenge, mean SR_L increased by $652 \pm 73\%$ and $657 \pm 56\%$ above baseline, without and following pretreatment with heparin, respectively ($p=NS$). Aerosol histamine produced a similar degree of bronchoconstriction whether histamine was dissolved in buffered saline or in heparin solution ($\Delta SR_L = 258 \pm 49\%$ vs $175 \pm 40\%$) (fig. 3).

EXAMPLE 5

Effect of inhaled heparin on partial thromboplastin time.

In order to exclude any effect of inhaled heparin on PTT, venous blood (5 ml) was obtained before and 20 min after nebulization of heparin (1,000 units/kg) for analysis of PTT ($n=7$). The highest dose of inhaled heparin used in the study (1,000 units/kg) failed to modify the plasma partial thromboplastin time; mean values were 30 ± 2 and 31 ± 2 seconds before and after aerosol heparin ($p=NS$). In subsequent experiments, we have also observed that inhaled heparin (1,000 units/kg) in sheep does not alter PTT, when

studied immediately after nebulization, or 6-12 hours post-heparin. These findings suggested that the antiallergic action of heparin is probably related to non-anticoagulant properties of heparin.

EXAMPLE 6

Pharmacodynamics of heparin action. - In this investigation, we studied the pharmacodynamics of antiallergic action of heparin. SR_L was measured in 8 sheep allergic to Ascaris suum antigen, before and after inhalation challenge with antigen. On 4 different days, antigen challenge was repeated after pretreatment with aerosol heparin (1,000 units/kg) administered 20 min, 6 hrs, 12 hrs and 24 hrs prior to antigen challenge. SR_L (mean \pm SE) increased by $374 \pm 116\%$ above baseline with antigen alone ($p < 0.05$). Aerosol heparin attenuated the antigen effects in a time-dependent fashion. The peak inhibitory effect of aerosol heparin was observed at 20 min pretreatment, and the degree of inhibition decreased with time; SR_L values were $31 \pm 29\%$, $99 \pm 38\%$, $142 \pm 40\%$ and $306 \pm 60\%$ for 20 min, 6 hrs, 12 hrs, and 24 hrs pretreatments, respectively (fig. 4). From these pharmacodynamic studies we concluded that antiallergic effects of heparin are time-related and the peak effect of inhaled heparin is observed soon after administration.

EXAMPLE 7

In vitro effects of heparin on antigen-induced trachealis muscle contraction. - We have also tested the antiallergic actions of heparin by determining if heparin blocked immunologically-induced tracheal smooth muscle contraction in vitro. Tracheal smooth muscle was obtained from sheep allergic to Ascaris suum antigen, and was suspended in an organ bath containing warmed (39°C) oxygenated (95% O₂, 5% CO₂) Krebs-Henseleit buffer. Tissues were allowed to equilibrate for 1 hr at resting tension of 1 g. After the equilibration period, the tissues were treated with heparin (injection USP, Elkin-Sinn) at concentrations of 10, 100, or 1000 µg/ml (final concentration in the bath) dissolved in 10 µl DMSO. Two types of controls were used: vehicle (10 µl DMSO) treated tissues and tissues treated with the anti-asthma drug, nedocromil sodium (10⁻⁵M). Tissues were challenged, after a 30 min pretreatment, with 10, 30 and 100 µl of antigen (162,000 protein nitrogen units/ml). Contractions induced by antigen were expressed as a percentage of the contraction elicited by a maximally effective concentration of acetylcholine (100 mM). Antigen produced dose-dependent increases in tension, which were blocked by heparin and nedocromil sodium (fig. 5).

Neither heparin nor nedocromil sodium affect the maximum response to acetylcholine. Likewise, dose response curves to acetylcholine were unaffected by any

concentration of heparin used. The addition of the heparin preservative benzyl alcohol did not reverse acetylcholine-induced contractions or inhibit antigen-induced contractions in sheep tracheal smooth muscle, as has been observed in dog bronchi. These results support our in vivo findings and suggest that heparin blocks immunologically tracheal smooth muscle contraction without affecting agonist induced contraction. This action is similar to that of the anti-asthma drug nedocromil sodium and may be related to inhibition of mast cell mediator release.

EXAMPLE 8

Effect of heparin on exercise-induced bronchoconstriction in patients with asthma (n=3).

- Preliminary studies were conducted in 3 subjects with history of marked exercise-induced bronchoconstriction (EIB). These subjects were studied on 3 different days, 7 days apart. On day 1, after obtaining resting pulmonary function tests, the subjects were screened for EIB. The subjects exercised on a treadmill, with increasing speed and degree of inclination, until their heart rate reached 85% of predicted maximum. The achieved exercise work-load was then continued for 10 min. Throughout the study, the heart rate was monitored continuously with an EKG. Minute ventilation, estimated with a calibrated respiratory inductive plethysmograph, measuring specific airway conductance (SG_{aw}) before, immediately after the exercise, and serially every 5 min

for 30 min post-exercise.

After the initial screening day, the subjects were studied on 2 separate days, in a single-blind randomized fashion. The work-load estimated on the initial screening day was kept constant on the two test days. The subjects were pretreated with an aerosol (4ml) of either heparin (1,000 units/kg) or a placebo solution (0.01 ml/ml benzyl alcohol in bacteriostatic injection water). SG_{aw} was obtained before and 45 min after nebulization of heparin or placebo solution. Exercise challenge was then performed, as stated above, and measurements of SG_{aw} were obtained immediately after exercise and every 5 min for 30 min post-exercise. On both test days, the heart rate and minute ventilation were monitored as on a control day.

These studies in 3 subjects demonstrated that inhaled heparin (at a dose which blocked compound 48/80 and antigen-induced bronchoconstriction in sheep, i.e. 1,000 units/kg) markedly attenuated EIB (fig. 6). Both the magnitude as well as the duration of EIB were attenuated.

It has thus been shown that there are provided a method and compositions which achieve the various objects of the invention and which are will adapted to meet the conditions of practical use.

As various possible embodiments might be made of the above invention, and as various changes might be made

in the embodiments set forth above, it is to be understood that all matters herein described are to be interpreted as illustrative and not in a limiting sense.

What is claimed as new and desired to be protected by Letters Patent is set forth in the following claims.

I CLAIM:

1. A method of treating a patient suffering from antigen-induced or exercise-induced asthma comprising the intrabronchial administration to the patient of an inhalant composition containing about 300 to about 2,000 units of heparin per kilogram of patient body weight in each dose.
2. A method according to claim 1 wherein said patient is suffering from antigen-induced asthma.
3. A method according to claim 2 wherein said patient receives one to about four doses of said composition per day.
4. A method according to claim 3 wherein the patient receives from two to four doses of said composition per day.
5. A method according to claim 1 wherein said patient is suffering from exercise-induced asthma.
6. A method according to claim 5 wherein said patient receives one dose of said composition from about thirty minutes to about three hours before exercise.
7. A method according to claim 6 wherein said patient receives an additional dose of said composition during or after exercise.
8. A method according to claim 1 wherein said composition contains about 500 to about 1,000 units/kg of heparin per dose.

9. A method according to claim 8 wherein said composition contains about 1,000 units/kg of heparin per dose.

10. A method according to claim 1 wherein said heparin is commercial heparin.

11. A method according to claim 1 wherein said heparin is a low molecular weight heparin or heparin fragment having an average molecular weight of about 1,500 to about 6,000.

12. A method according to claim 11 wherein said low molecular weight heparin or heparin fragment has an average molecular weight of about 4,000 to about 5,000.

13. A method according to claim 1 wherein said heparin is a partially N-desulfated heparin or other non-anticoagulant heparin.

14. A method according to claim 13 wherein said partially N-desulfated heparin has from about 25 to about 85% of the N-sulfate groups of commercial heparin.

15. A method according to claim 1 wherein said inhalant composition is a solution of heparin in an aqueous, pharmaceutically acceptable inhalant vehicle.

16. A method according to claim 15 wherein said vehicle is isotonic saline or bacteriostatic water.

17. A method according to claim 15 wherein said composition includes about 5,625 to about 37,500 units of heparin per milliliter.

18. A method according to claim 15 wherein said composition is administered by means of a pump or squeeze-actuated nebulizer.

19. A method according to claim 15 wherein said composition further includes an aerosol propellant and is administered via a metered aerosol dose inhaler.

20. A method according to claim 1 wherein said inhalant composition comprises a powdered preparation of heparin intermixed with an inert powder acceptable for intrabronchial administration.

21. A method according to claim 20 wherein said inert powder is lactose.

22. A method according to claim 20 wherein said powder is administered via an aerosol dispenser.

23. A method according to claim 20 wherein said powder is administered from a breakable capsule.

24. A pharmaceutical composition for treatment of a patient suffering from antigen-induced or exercise-induced asthma comprising about 300 to about 2,000 units of heparin per kilogram of patient body weight in each dose in a pharmaceutically acceptable inhalant vehicle.

25. A composition according to claim 24 which comprises about 500 to about 1,000 units/kg of heparin per dose.

26. A composition according to claim 25 which comprises about 1,000 units/kg of heparin per dose.

27. A composition according to claim 24 wherein said heparin is commercial heparin.

28. A composition according to claim 24 wherein said heparin is a low molecular weight heparin or heparin fragment having an average molecular weight of about 1,500 to about 6,000.

29. A composition according to claim 28 wherein said low molecular weight heparin or heparin fragment has an average molecular weight of about 4,000 to about 5,000.

30. A composition according to claim 24 wherein said heparin is a partially N-desulfated heparin or other non-anticoagulant heparin.

31. A composition according to claim 30 wherein said partially N-desulfated heparin has from about 25 to about 85% of the N-sulfate groups of commercial heparin.

32. A composition according to claim 24 wherein said vehicle is an aqueous liquid.

33. A composition according to claim 32 wherein said liquid is isotonic saline or bacteriostatic water.

34. A composition according to claim 32 which includes about 5,625 to about 37,500 units of heparin per millimeter.

35. A composition according to claim 32 which additionally includes an aerosol propellant.

36. A composition according to claim 24 wherein said vehicle is a powder.

37. A composition according to claim 36 wherein said powder is lactose.

1/7

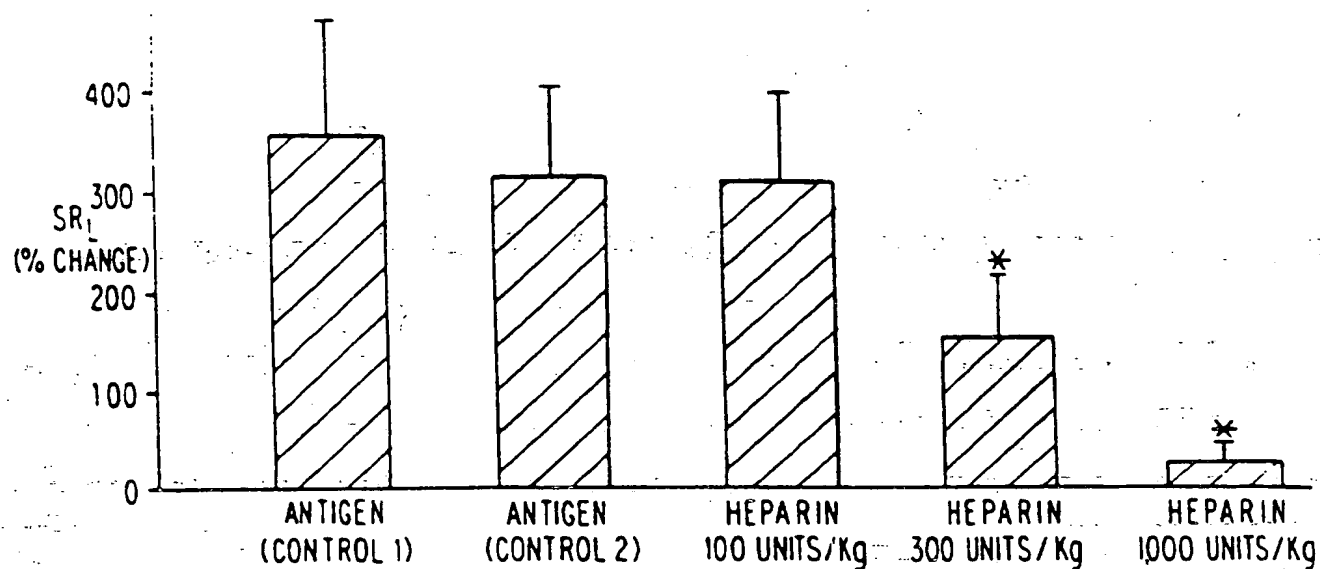


FIG. 1

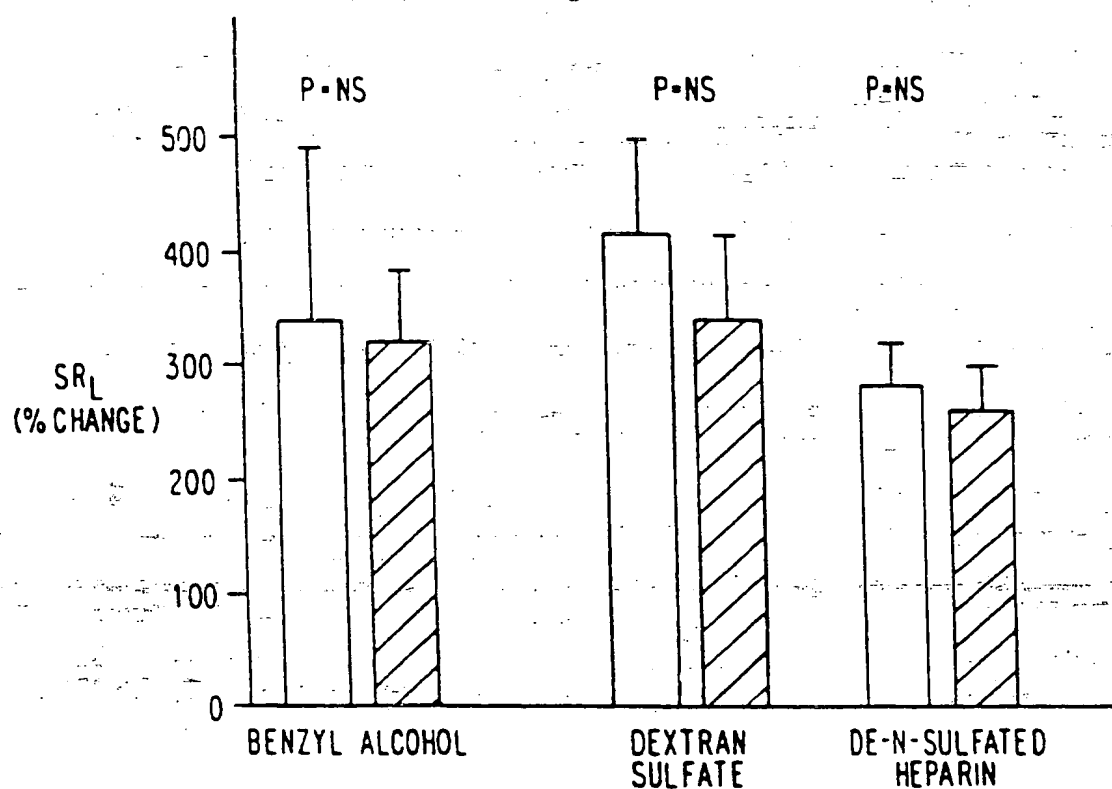
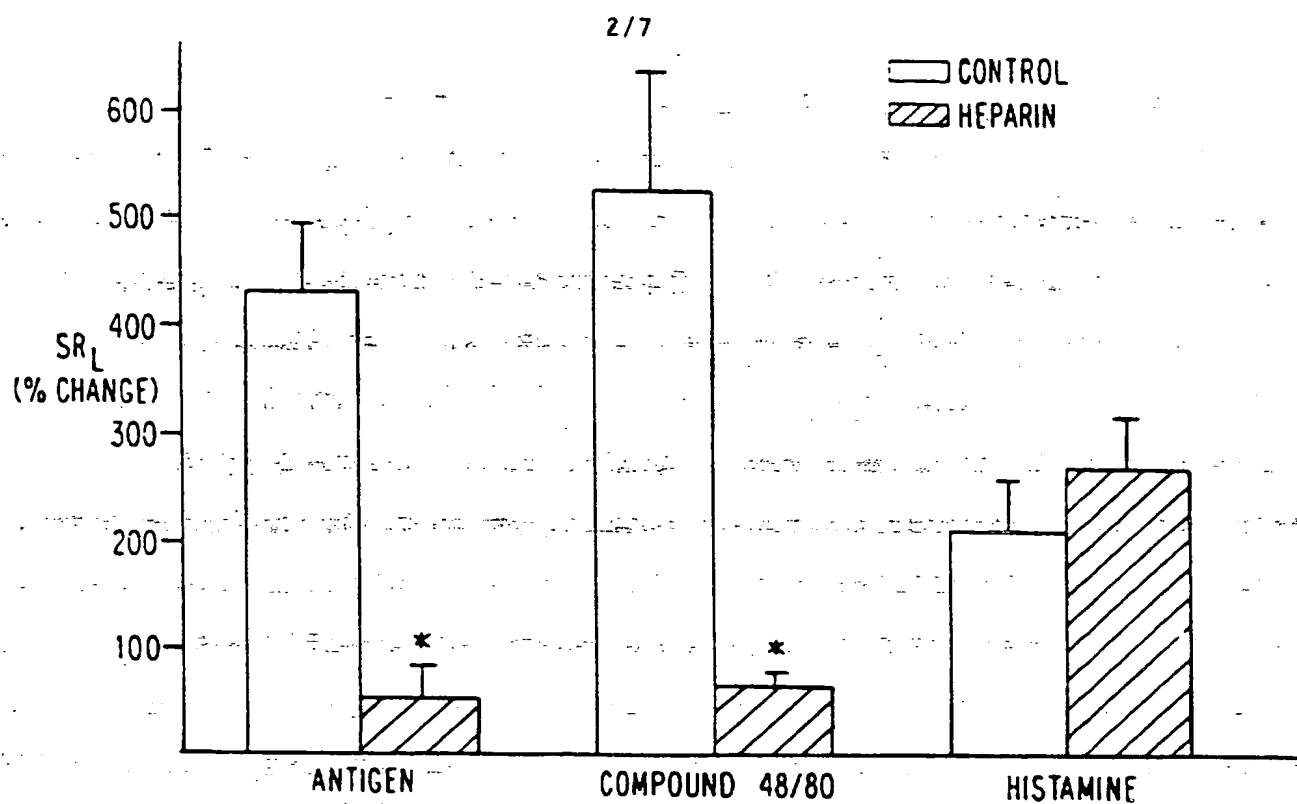
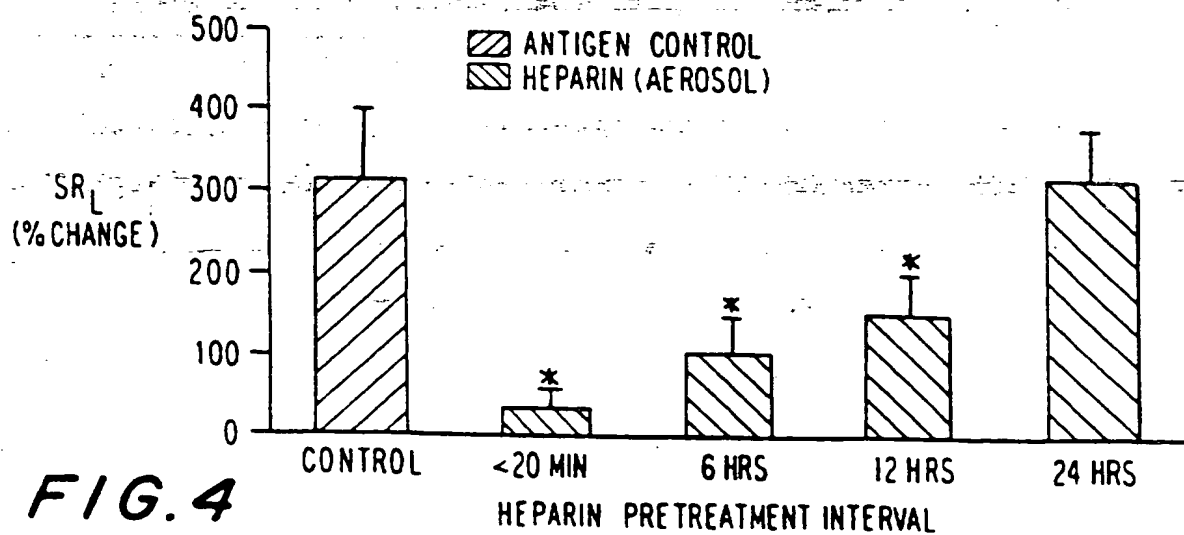


FIG. 2

**FIG. 3****FIG. 4**

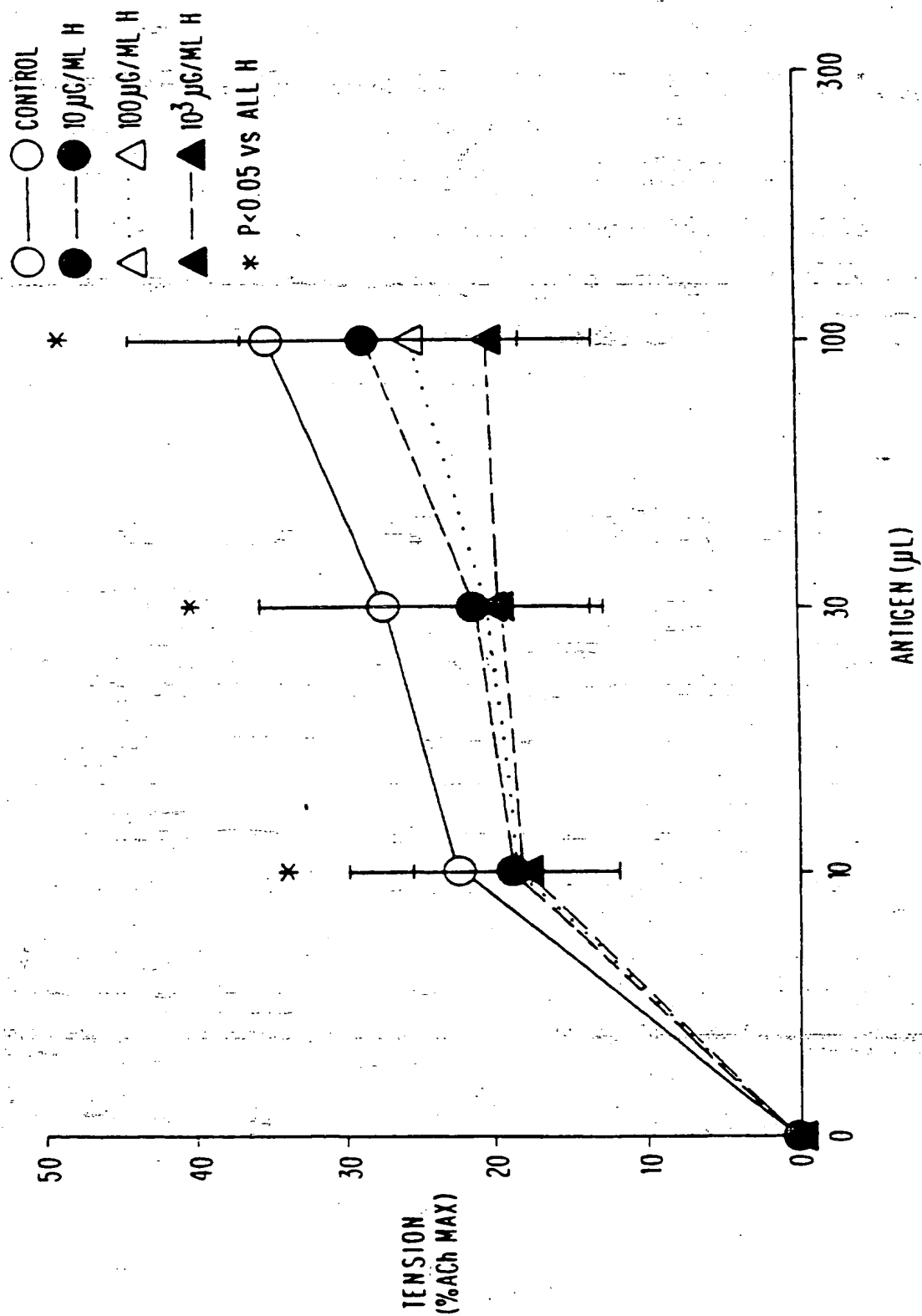


FIG. 5A

4 / 7

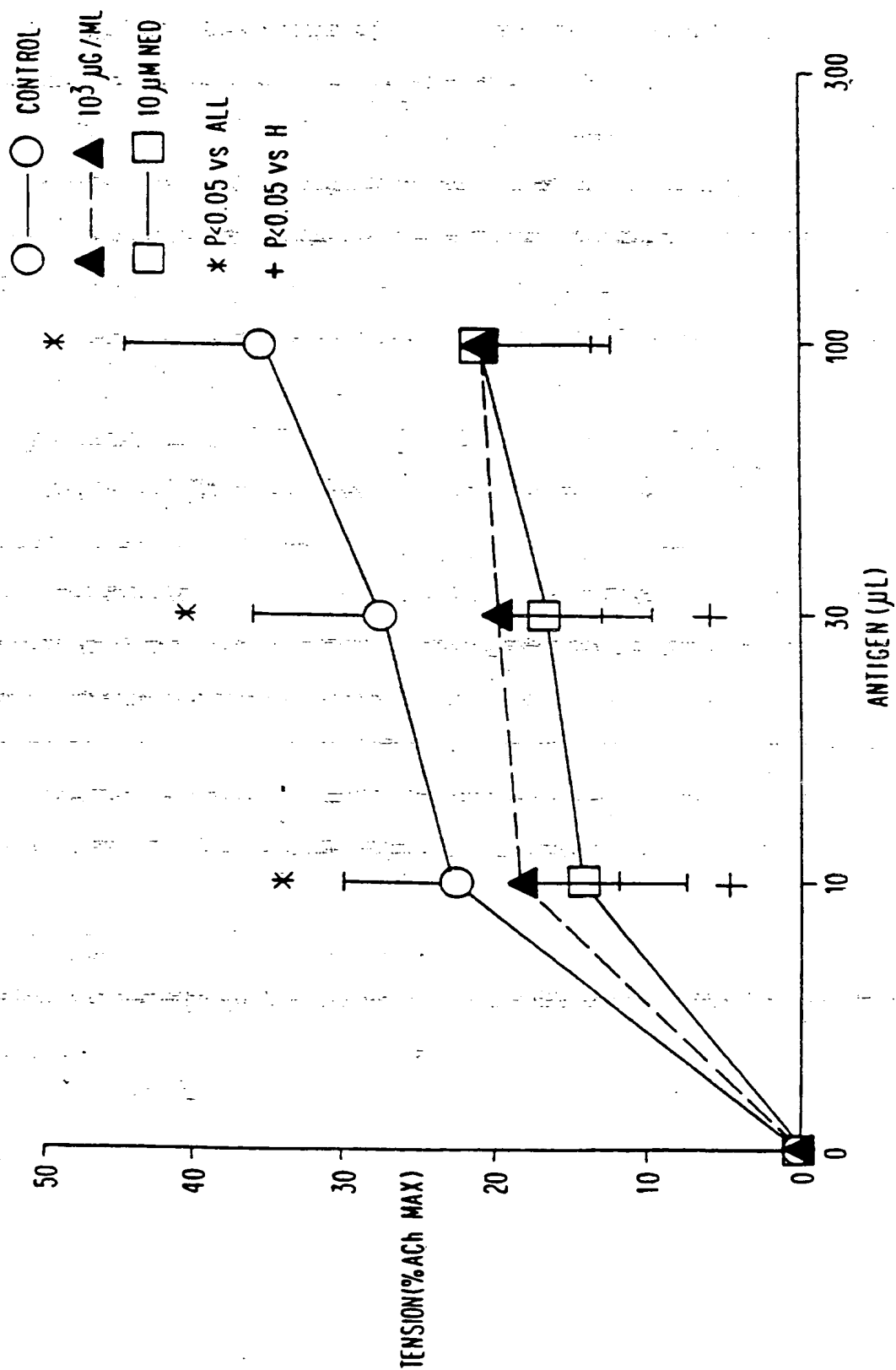


FIG. 5B

5 / 7

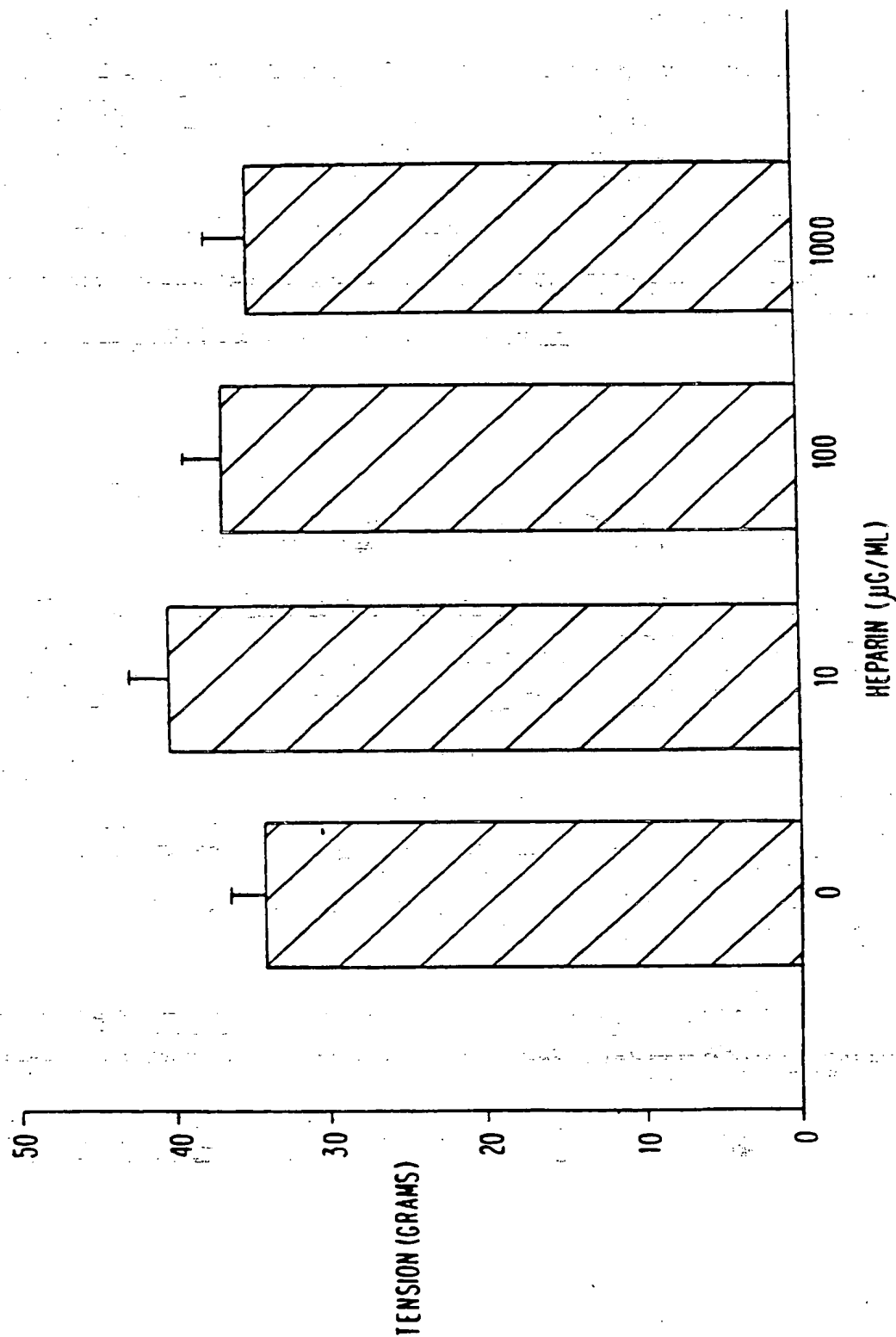


FIG. 5C

6 / 7

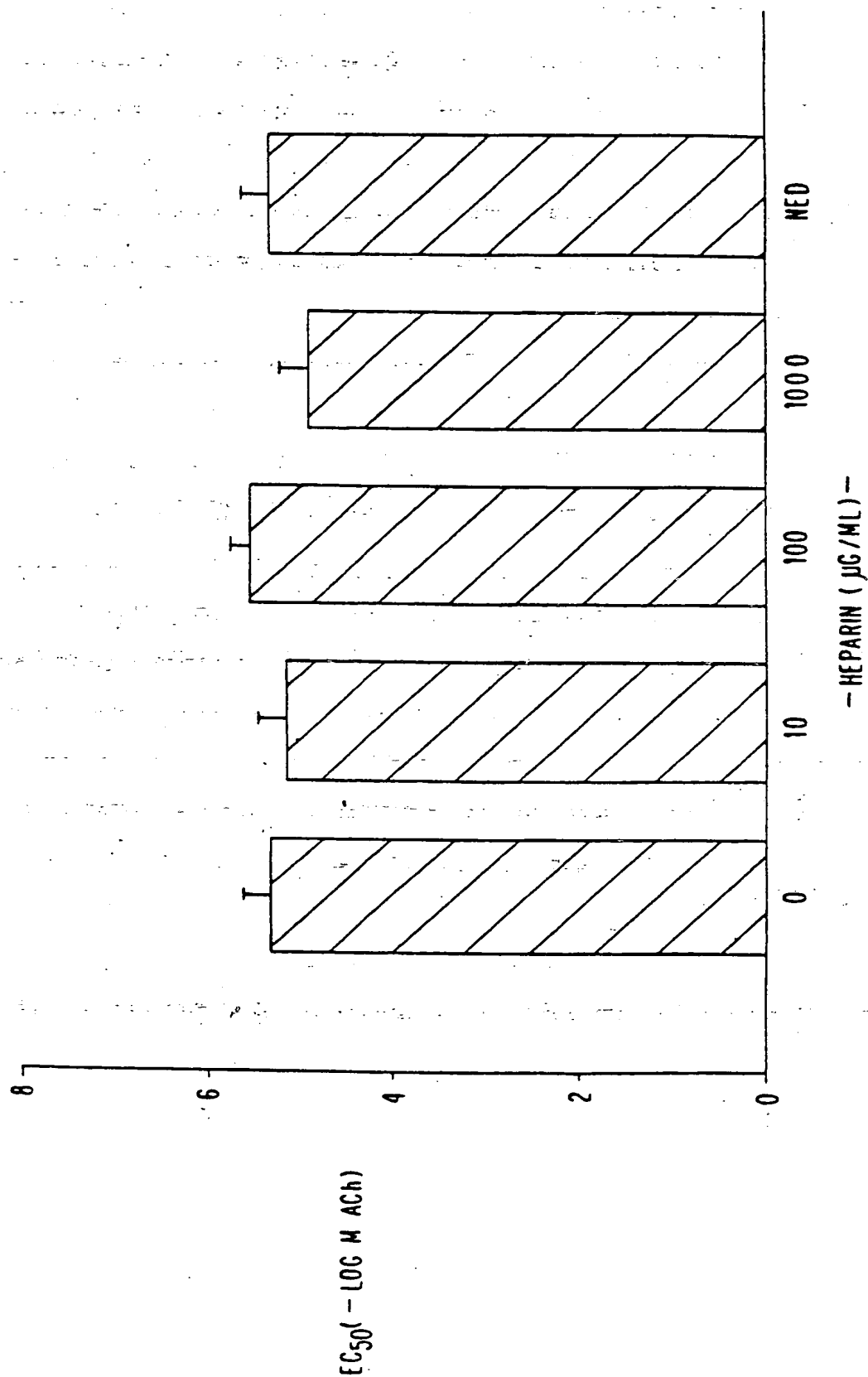


FIG. 5D

9/7

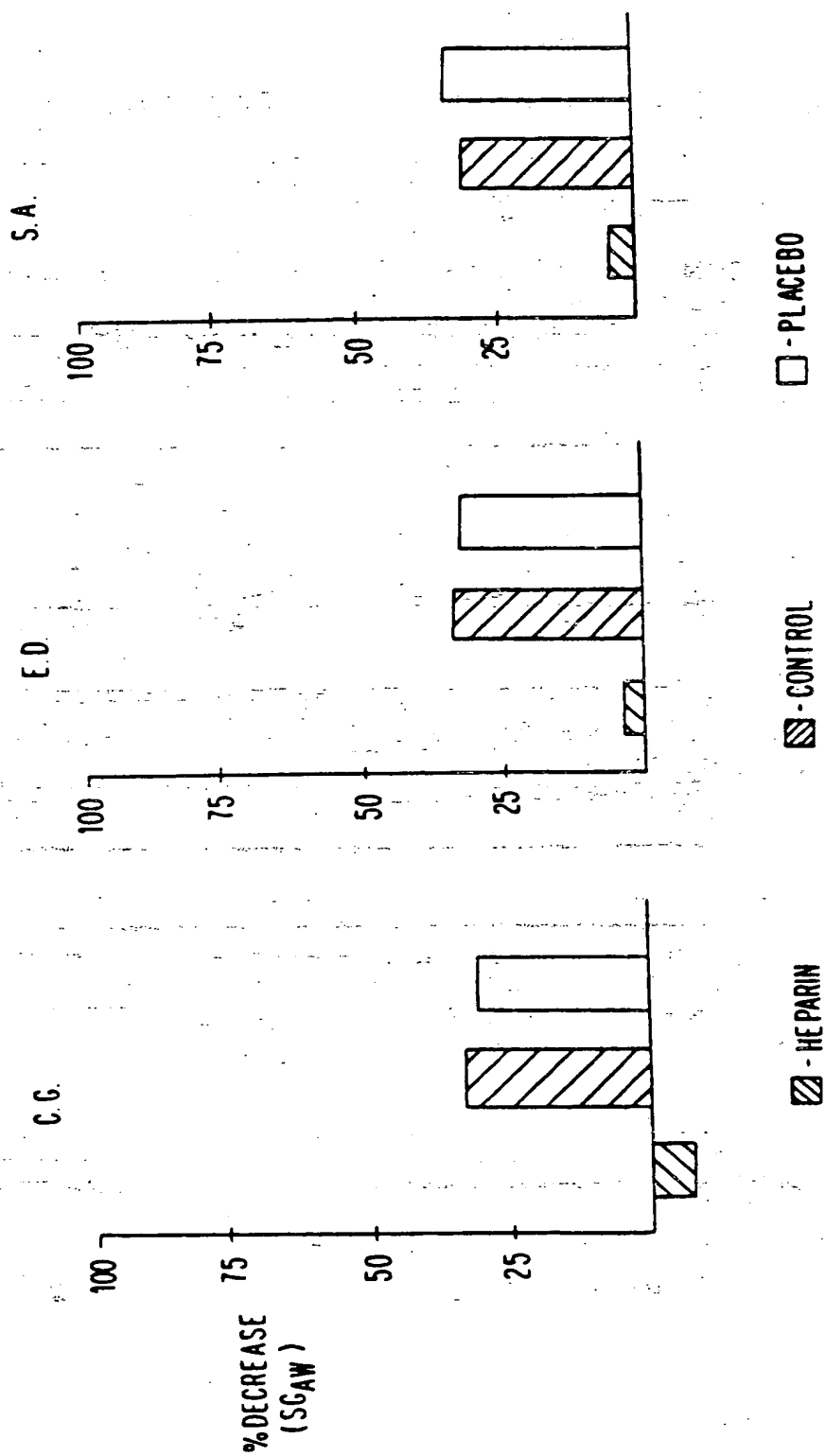


FIG. 6

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02880

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A61K-9/12, 31/725

US CL :514/54, 56; 536/21; 424/434, 488, 46

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/54, 56; 536/21; 424/434, 488, 46

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS Online search terms: heparin, heparinoid, nasal?, inhalant, aerosol, nebulizer, asthma.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	VUTR. BOLES, Vol. 26, no. 6, issued 1987, Petrov et al, "Inhalation treatment with low doses of heparin in bronchial asthma patients," abstract.	1-10, 15-16, 19-26, and 32-37
X	BIOLOGICAL ABSTRACTS, Vol. 85, no. 5, issued 01 March 1988, Ono et al, "Studies on Heparin in Allergic Reactions: 3. THERapeutic Use of Heparin in Bronchial Asthma," abstract no. 51021, Okayama Igakkai Zasshi, 99(5/6):559-568.	1-10, 15-16, 19-26, and 32-37
X	JP, A, 3-169821, (KISSEI PHARM KK) 23 July 1991, abstract.	1-37

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents: *A* document defining the general state of the art which is not considered to be part of particular relevance *E* earlier document published on or after the international filing date *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) *O* document referring to an oral disclosure, use, exhibition or other means *P* document published prior to the international filing date but later than the priority date claimed	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to underpin the principle or theory underlying the invention *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art *Z* document member of the same patent family
--	--

Date of the actual completion of the international search

06 May 1993

Date of mailing of the international search report

13 May 1993

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. NOT APPLICABLE

Authorized officer

NANCY S. HUSARIK

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US93/02880

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Chemical Abstracts, Vol. 113, no. 3, issued 16 July 1990, Landyshev et al, "Method of Treating Respiratory Insufficiency in Patients with Nonspecific Pulmonary Disease," see page 62, abstract no. 17897x.	24-27 and 32-37
X	Chemical Abstracts, Vol. 90, issued 1979, Moreau et al, "Pharmaceutical Based on Heparin for Tracheobronchial and Alveolar Administration," see page 312, abstract no. 43816w.	24-27 and 32-37
X	Chemical Abstracts, Vol. 102, issued 1985, Breitenstein et al, "Heparin Formulation Containing a Surfactant with Action on the Mucous Membranes of the Mouth, Nose, and/or the Throat," see page 352, abstract no. 154804b.	24-27 and 32-37
X	Chemical Abstracts, Vol. 108, issued 1988, Brown et al, "Dimethyl Sulfoxide with Heparin in the Treatment of Smoke Inhalation Injury," see page 69, abstract no. 198373p, J. Burn Care Rehabil. 9(1):22-5.	24-27 and 32-37
X	US, A, 5,037,810 (SALIBA, JR) 06 August 1991, see entire document.	24-27 and 32-37
X	Chemical Abstracts, Vol. 109, issued 1988, Johansen et al, "Nasal Pharmaceuticals Containing Low-Molecular Weight Heparin and a Fusidate," see page 370, abstract no. 116061u.	28-31
X	US, A, 5,032,679 (BRANDLEY ET AL) 16 July 1991, see entire document.	1, 11-14, and 28-31

THIS PAGE BLANK (USPTO)